

# Isolation of Non-Cytopathic (NCP) VSV Mutants

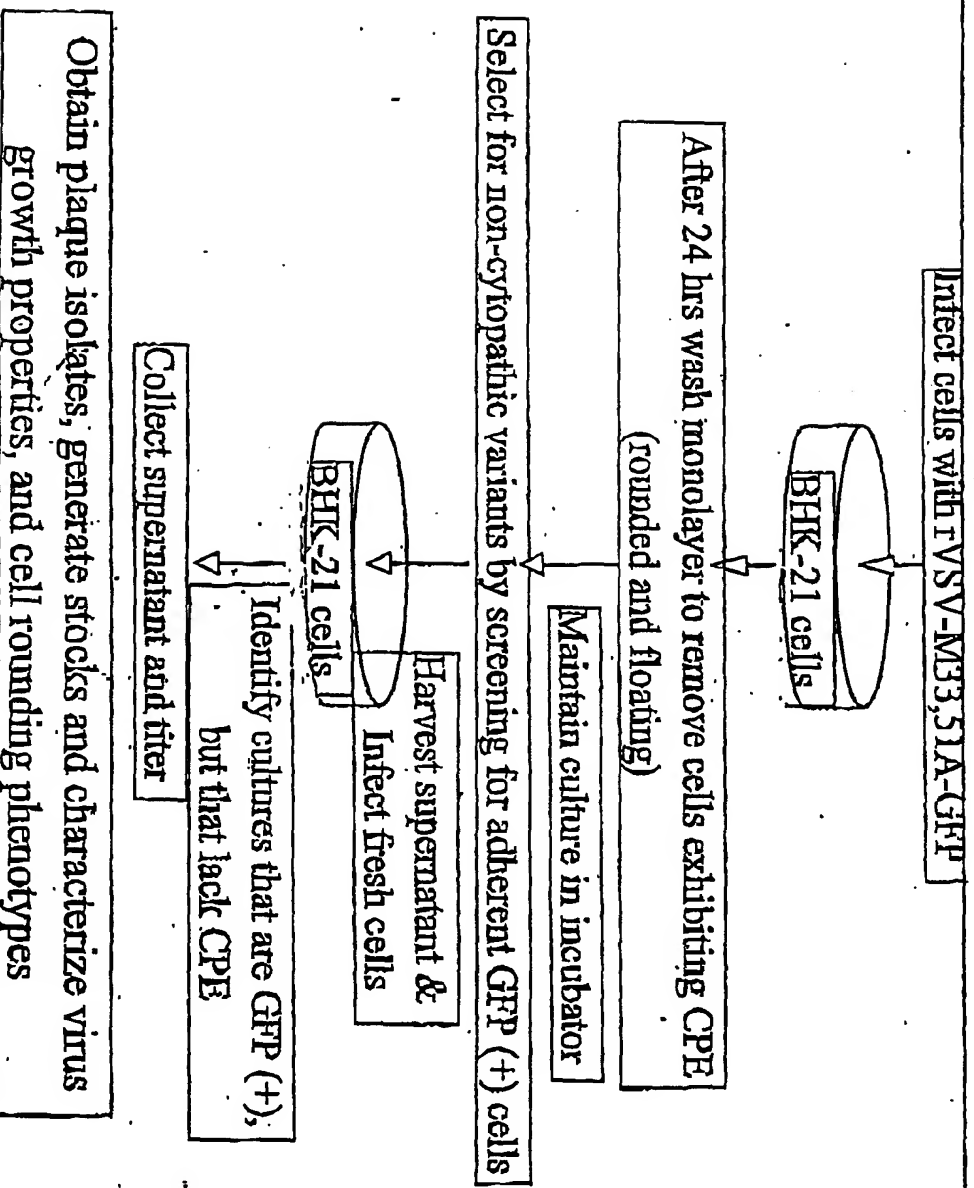
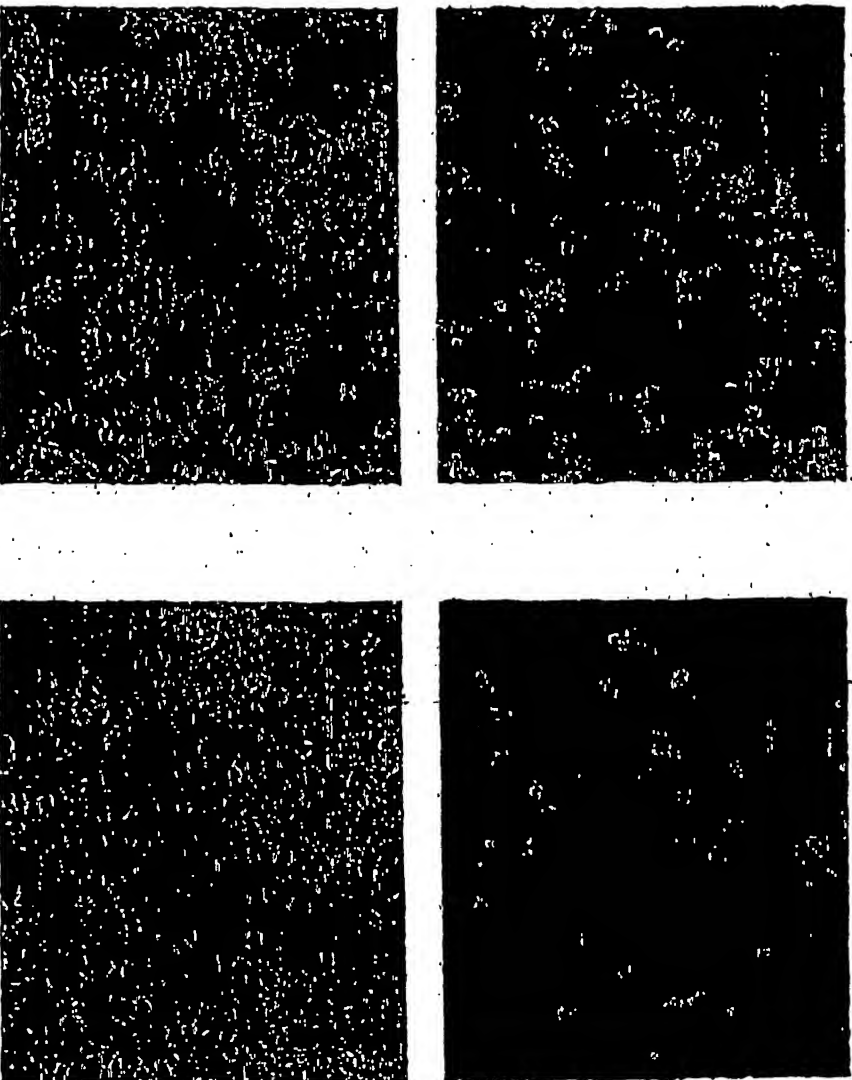


Figure 1

# Cell Rounding Phenotypes of M Protein Mutants of VSV



# Isolation and Sequencing of NCP-M Protein Mutant cDNAs

Purify NCP virus from culture supernatants of  
plaques purified NCP isolates



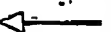
Isolate genomic RNA and perform RT-PCR using M gene specific primers



Subclone cDNAs into plasmid pBS-SK+



Sequence individual clones and identify mutations present in M<sub>NCP</sub> cDNAs



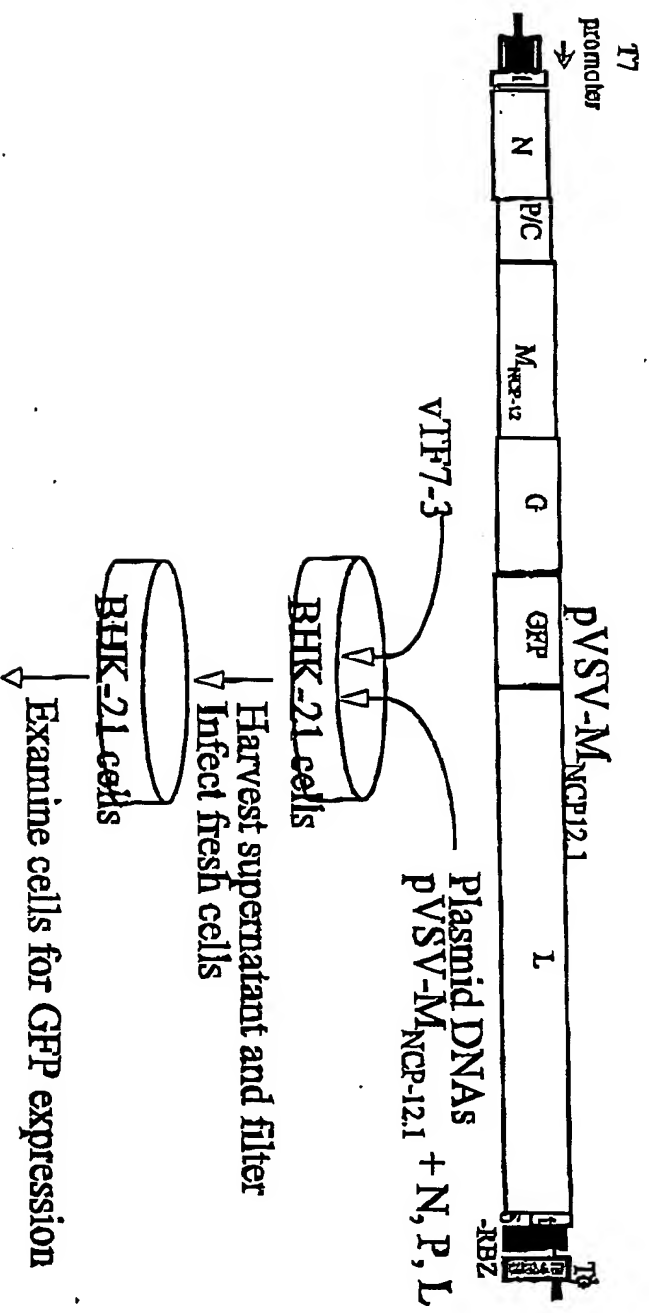
NCP mutations are M33A; M51A; T133A; & S226G

Designated M<sub>NCP12.1</sub>

Subclone M<sub>NCP12.1</sub> into pVSV-FL(+)-2

Figure 3

# Recovery of rVSV-M<sub>NCP12.1</sub>



Collect supernatant and titer

Obtain plaque isolates, generate stocks and characterize growth properties, and cell roundness

Figure 4

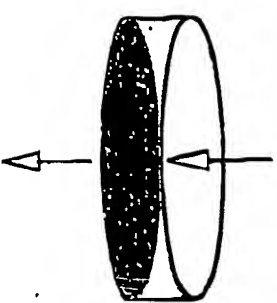
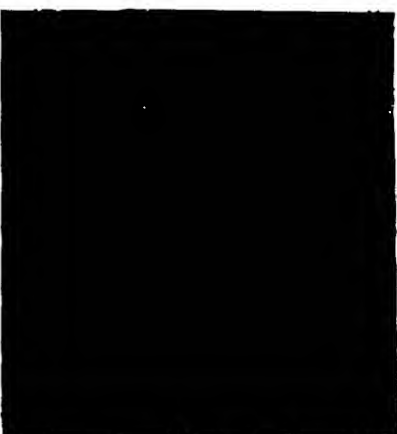
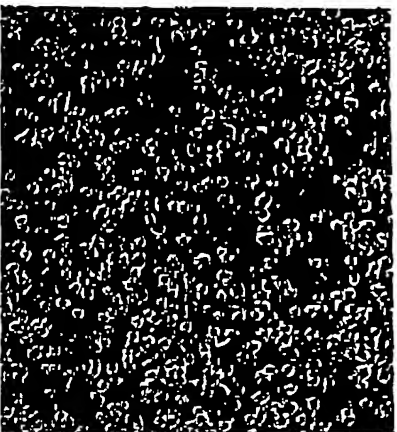
# rVSV/M<sub>NCP12.1</sub> is Defective in Cell Rounding

Phase Contrast

Anti-VSV N protein MAAb

Infect BHK-21 cells

wt rVSV



Examine @ 12 hrs p.i.

rVSV  
NCP-12.1

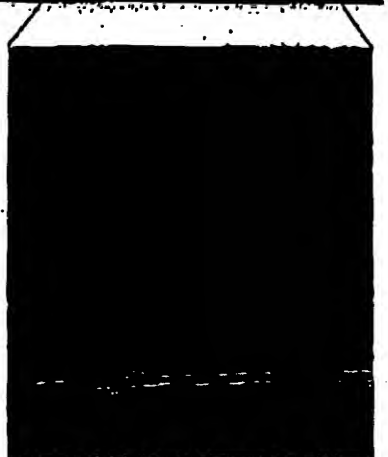
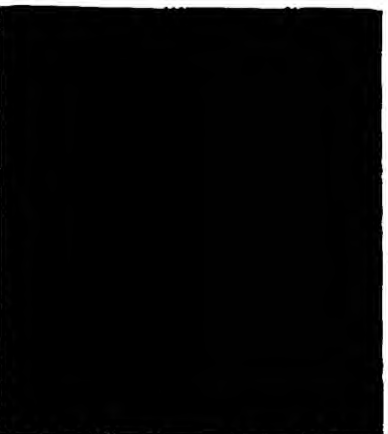
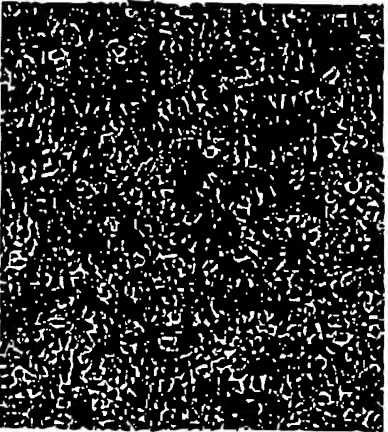


Figure 5

# Transient Expression of M<sub>NCP-12.1</sub>

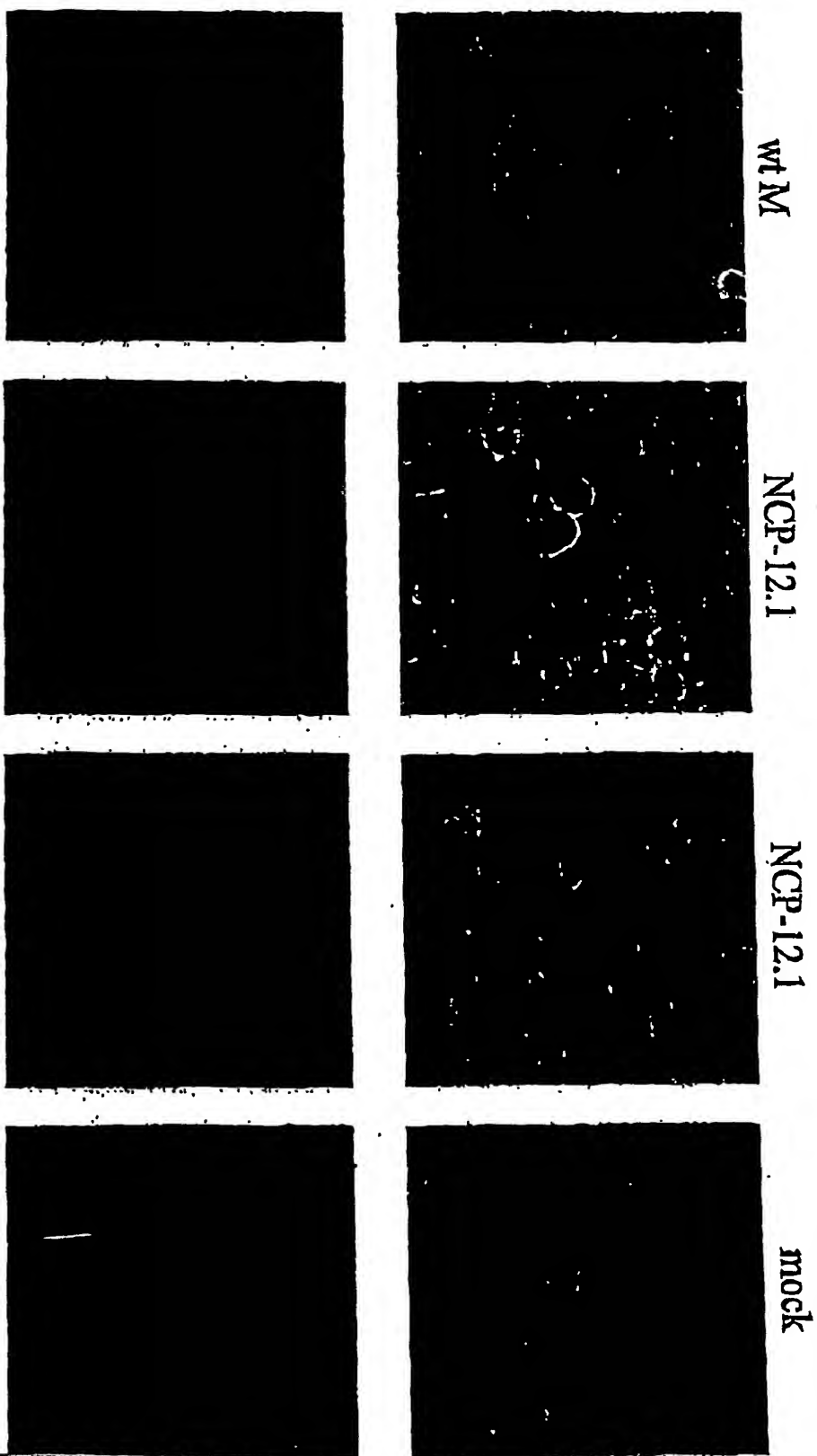


Figure 6

# rVSV/M<sub>NCP-12.1</sub> Infection of Different Cell Types

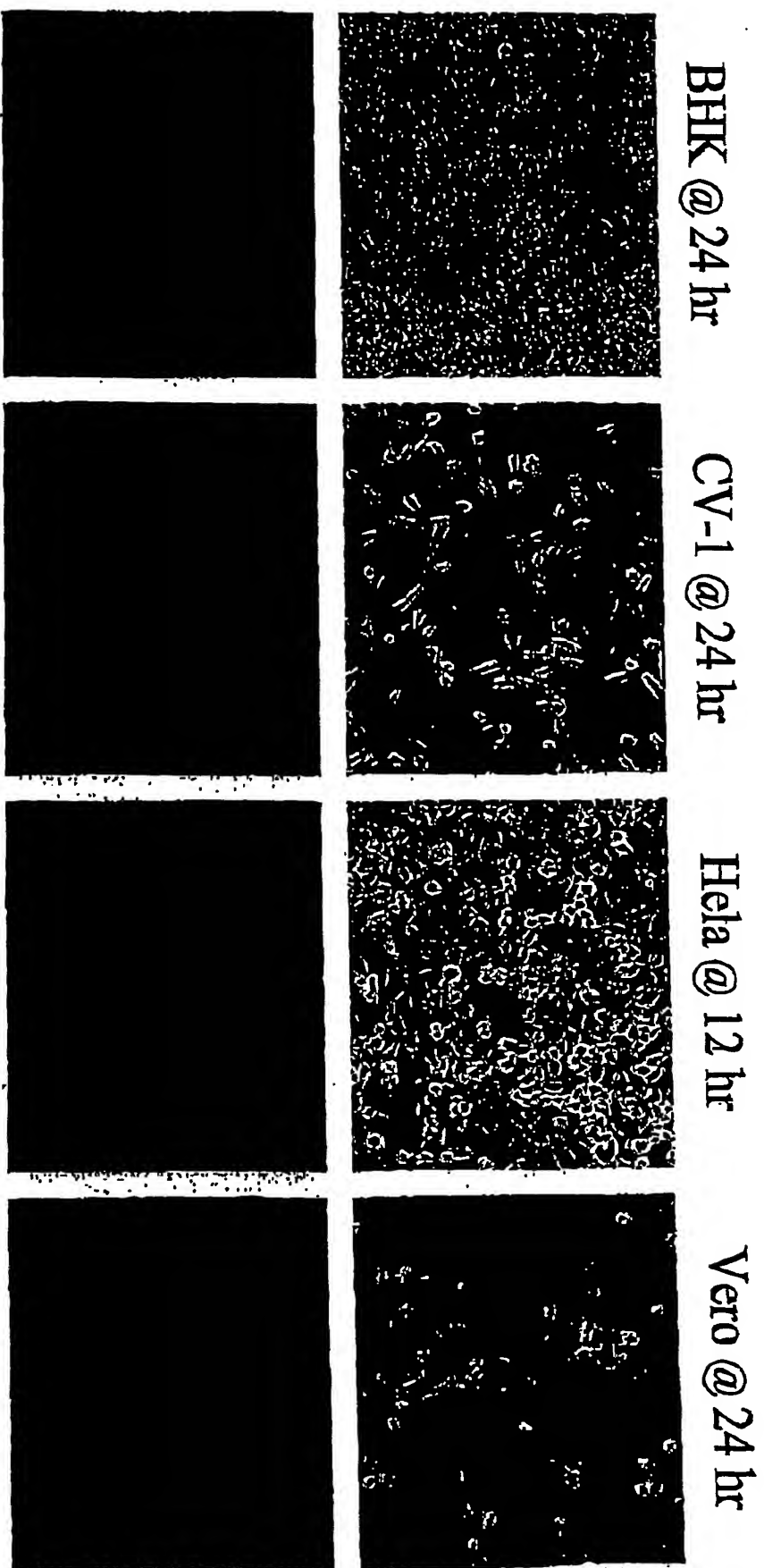


Figure 7

# Use of $M_{NCP-12.1}$ to Recover $\Delta M$ -VSV

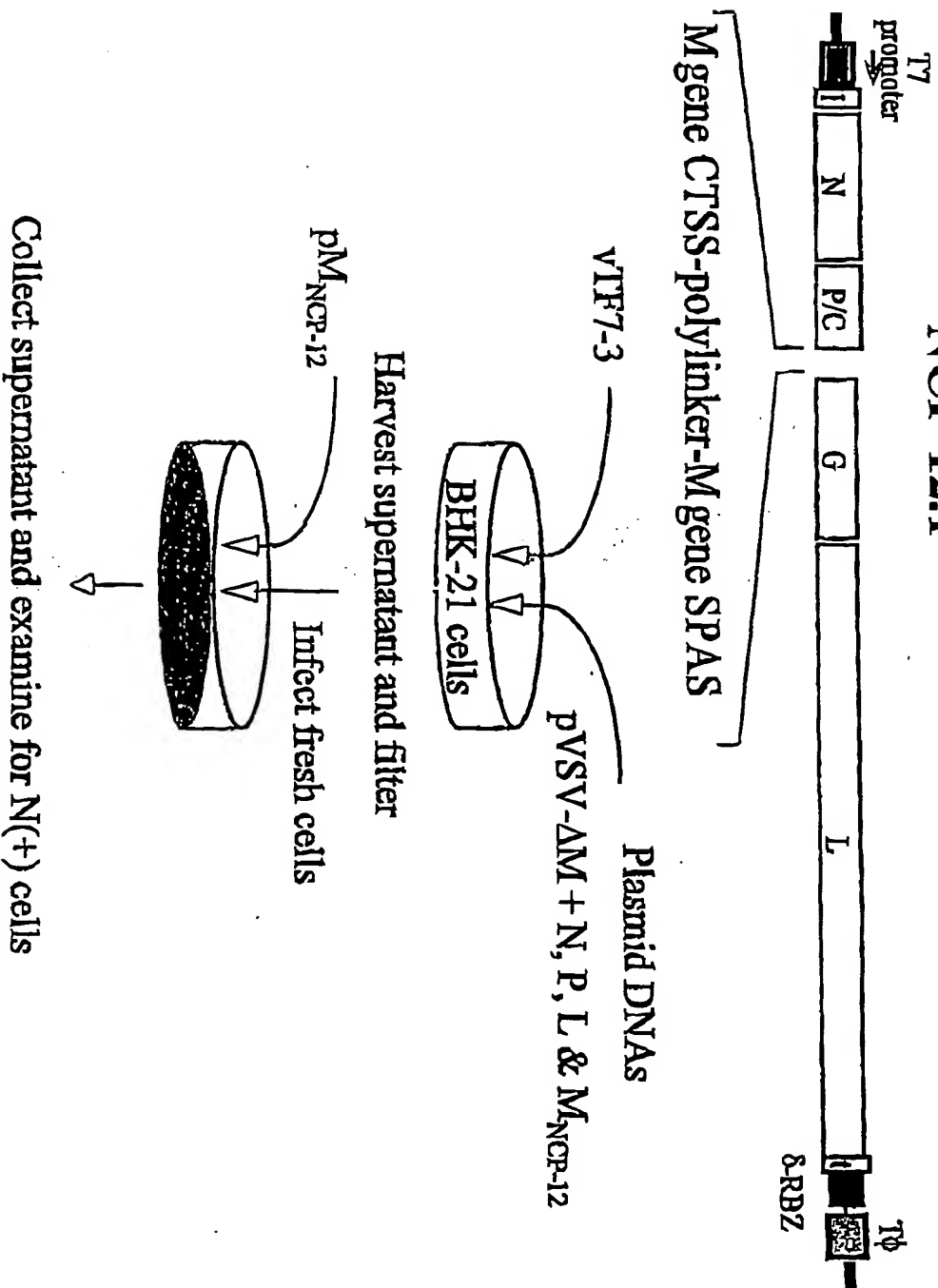
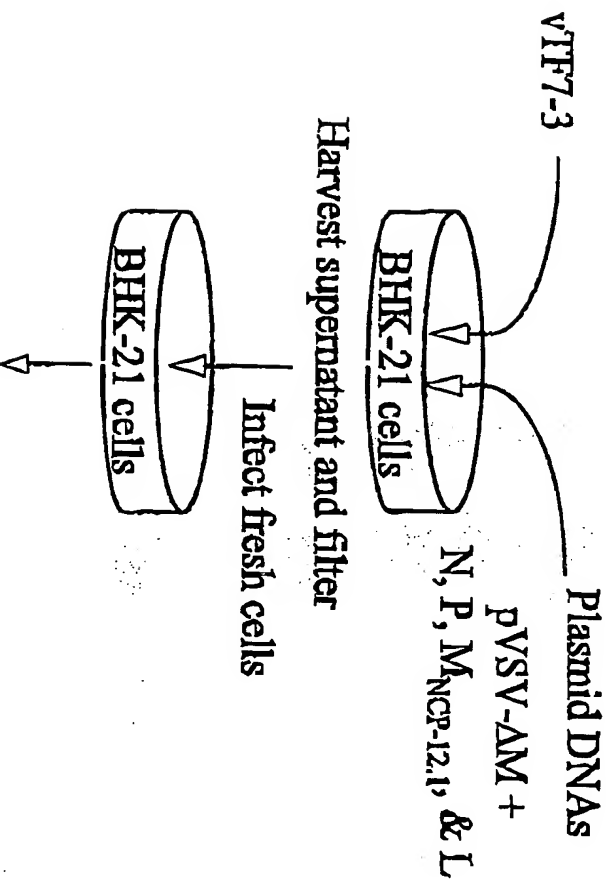


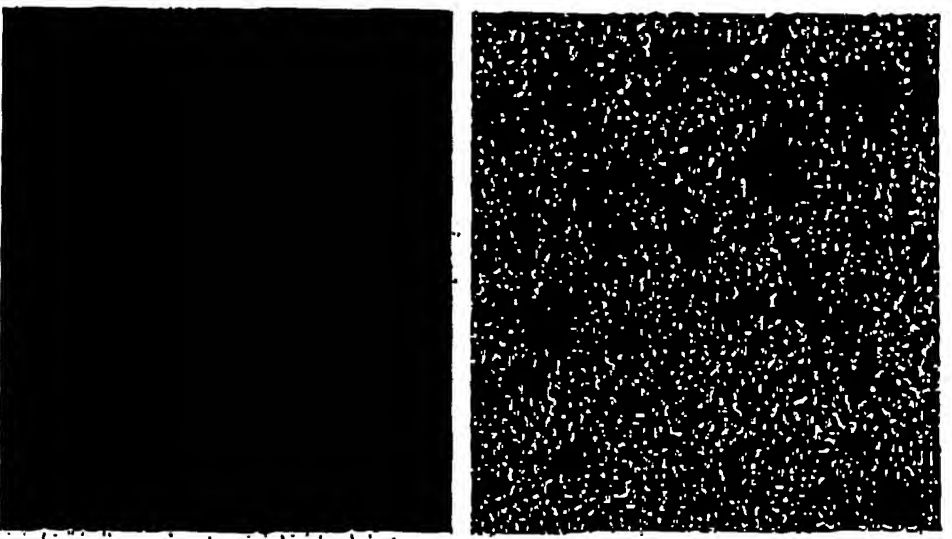
Figure 8

# Recovery of rVSV-ΔM



Examine for N protein expression

Figure 9



# Sample #176, 3 Days Post-infection

Infection of islet Prep #176 at day 3 post infection

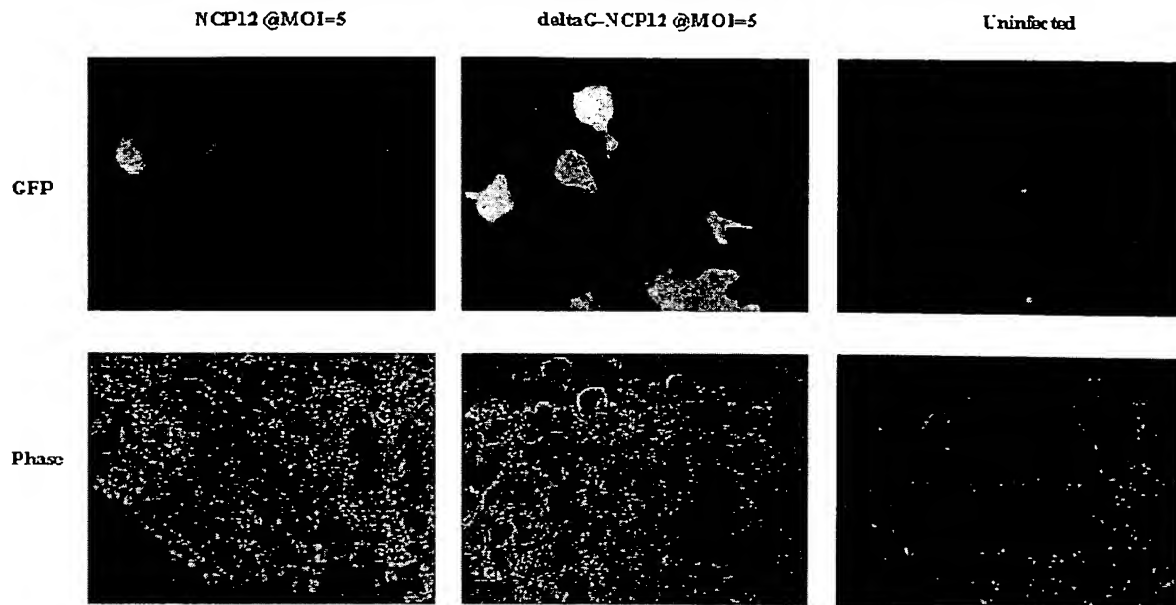


Figure 10

# Sample #163, 3 Days Post-infection

Infection of islet Prep#163 at day 3 post infection

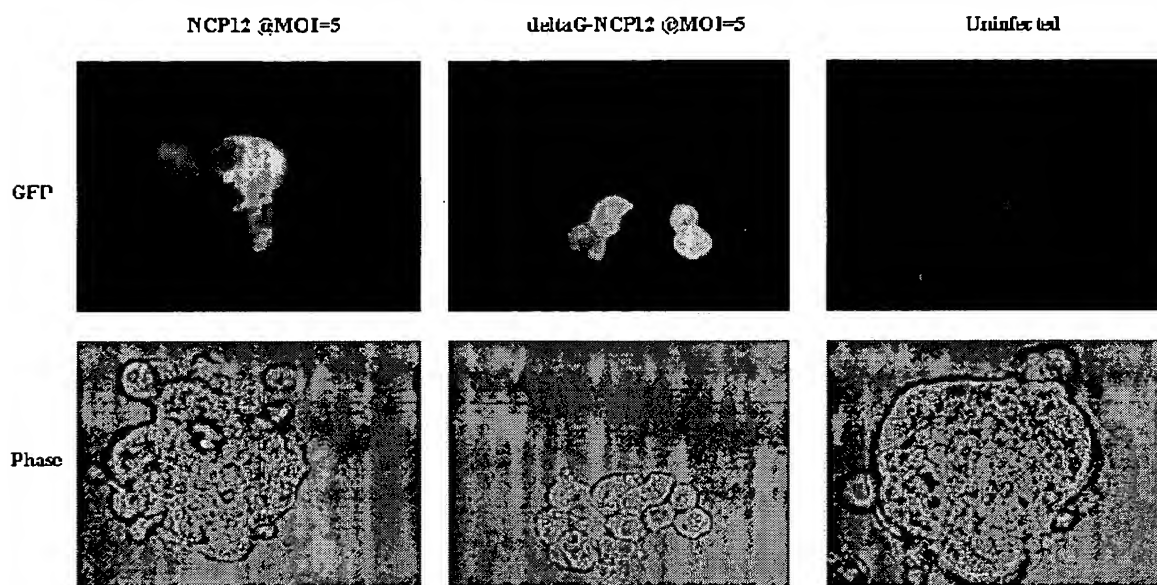


Figure 11

# Sample #176, 3 Days Post-infection

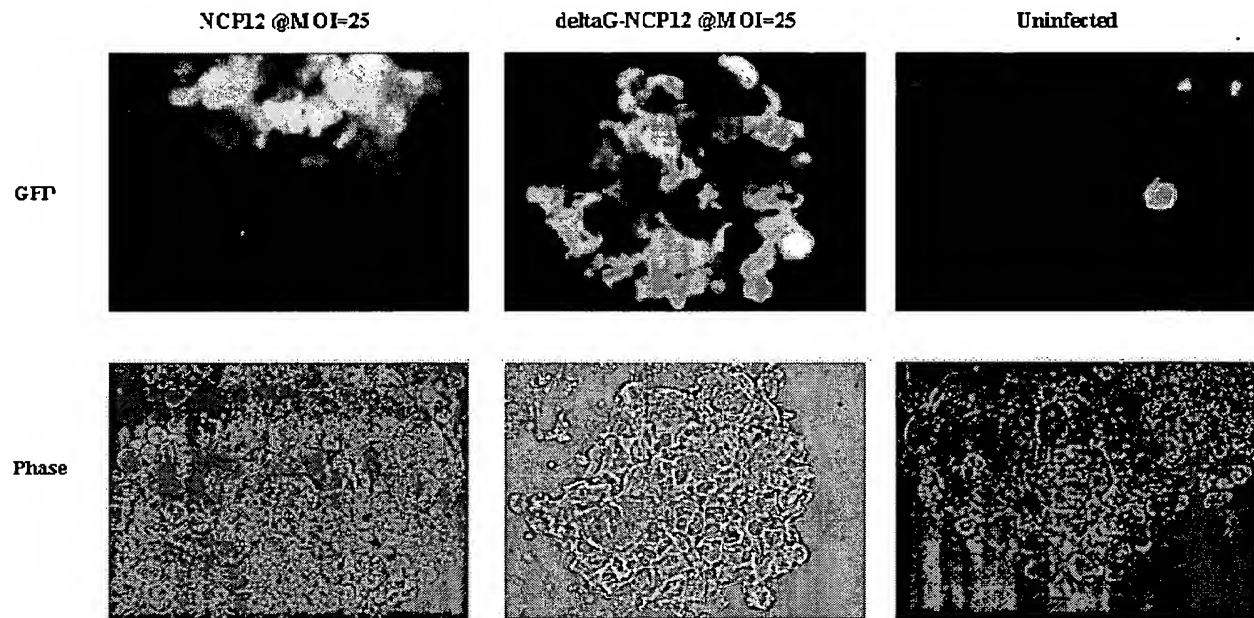


Figure 12

# Sample #163, 3 Days Post-infection

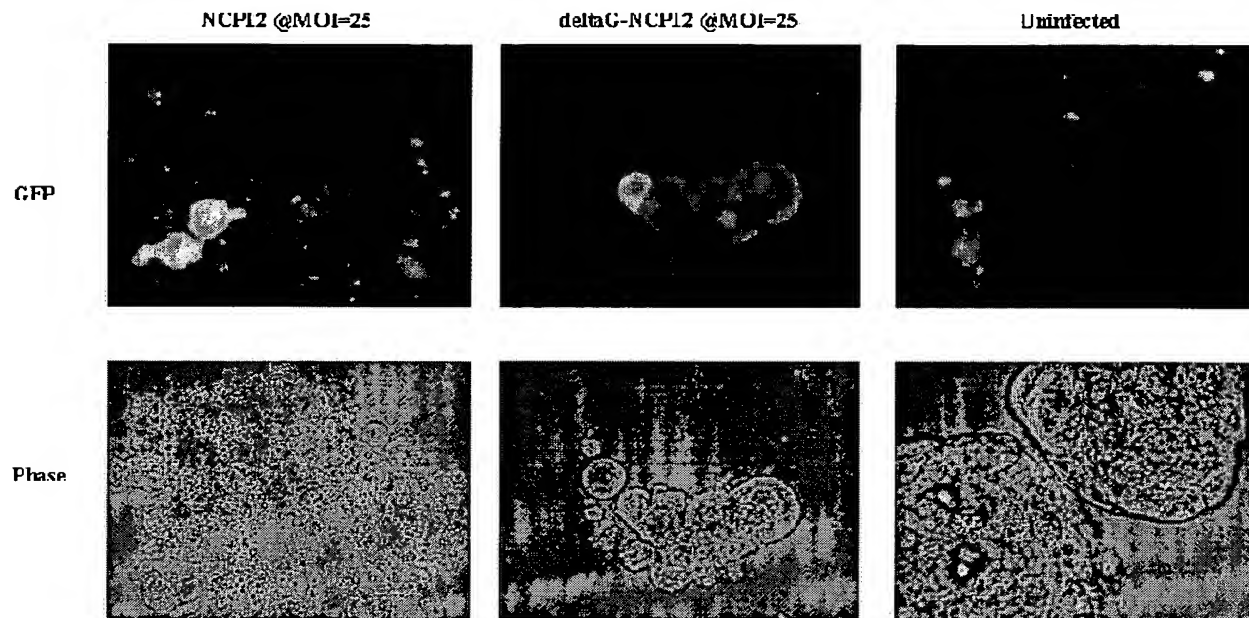


Figure 13

# Sample #176, 8 Days Post-infection

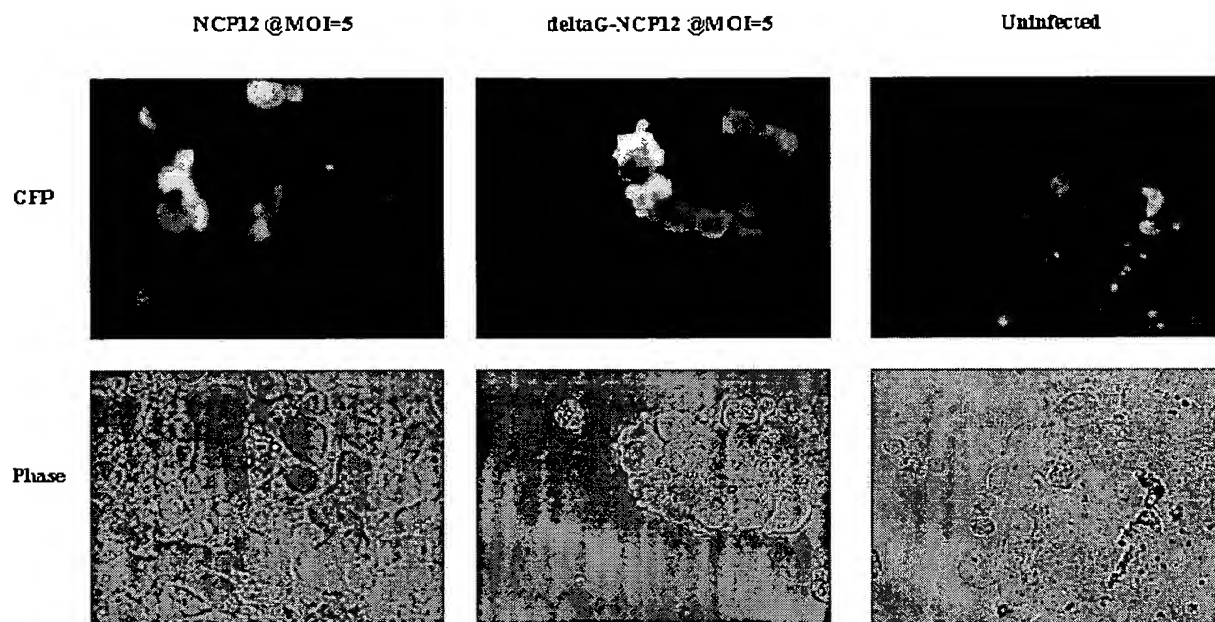


Figure 14

# Sample #176, 8 Days Post-infection

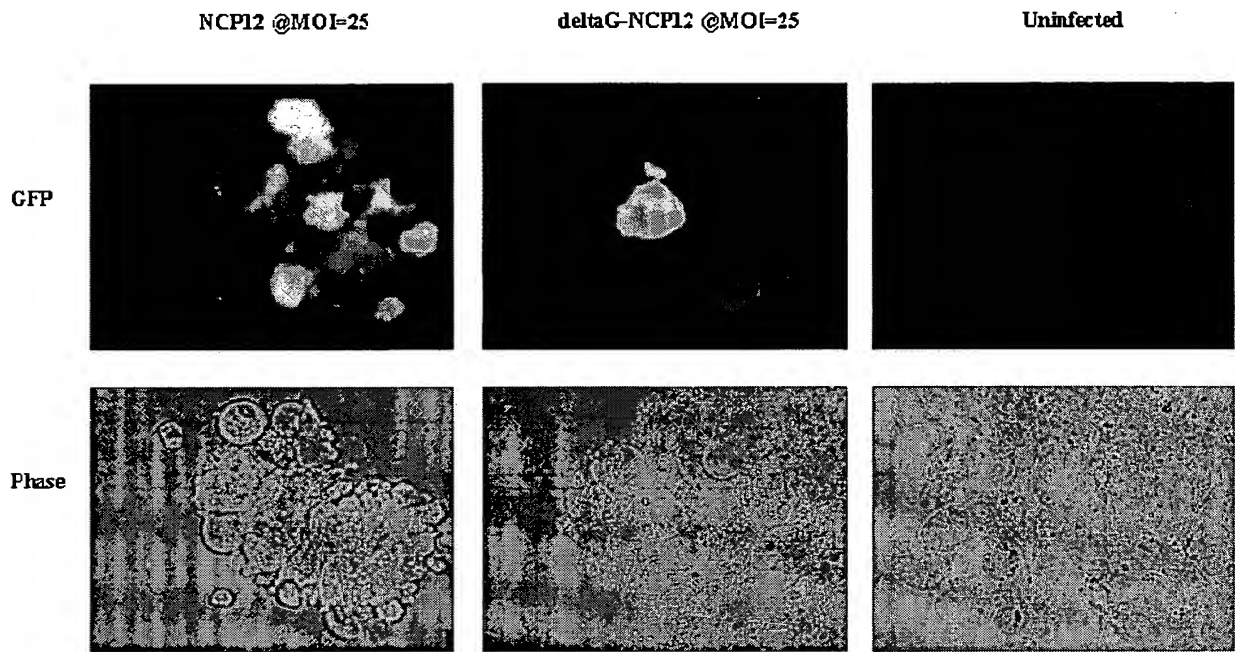


Figure 15

# Sample #163, 8 Days Post-infection

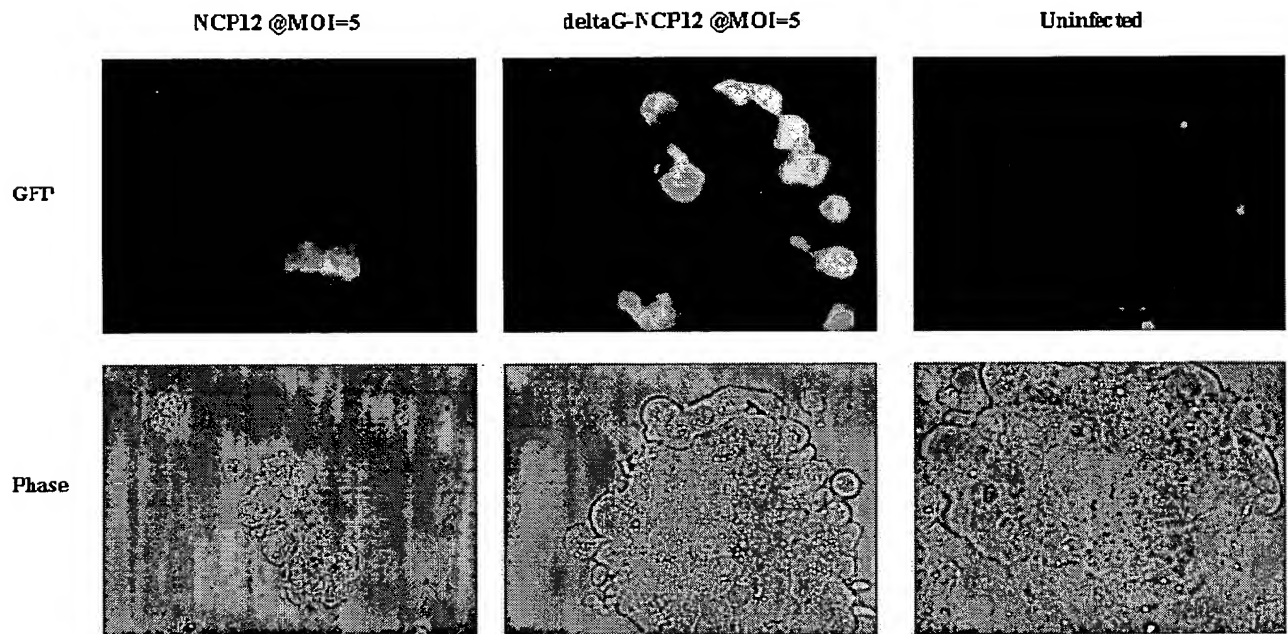


Figure 16

# Sample #163, 8 Days Post-infection

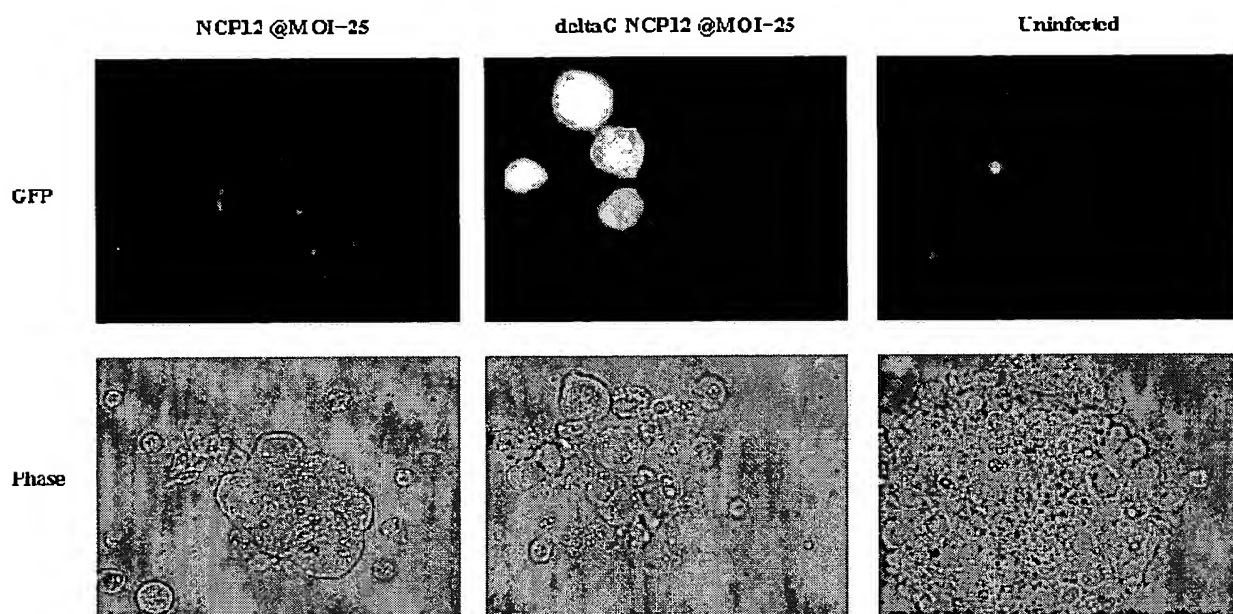


Figure 17

# Sample #176, 3 Days Post-infection

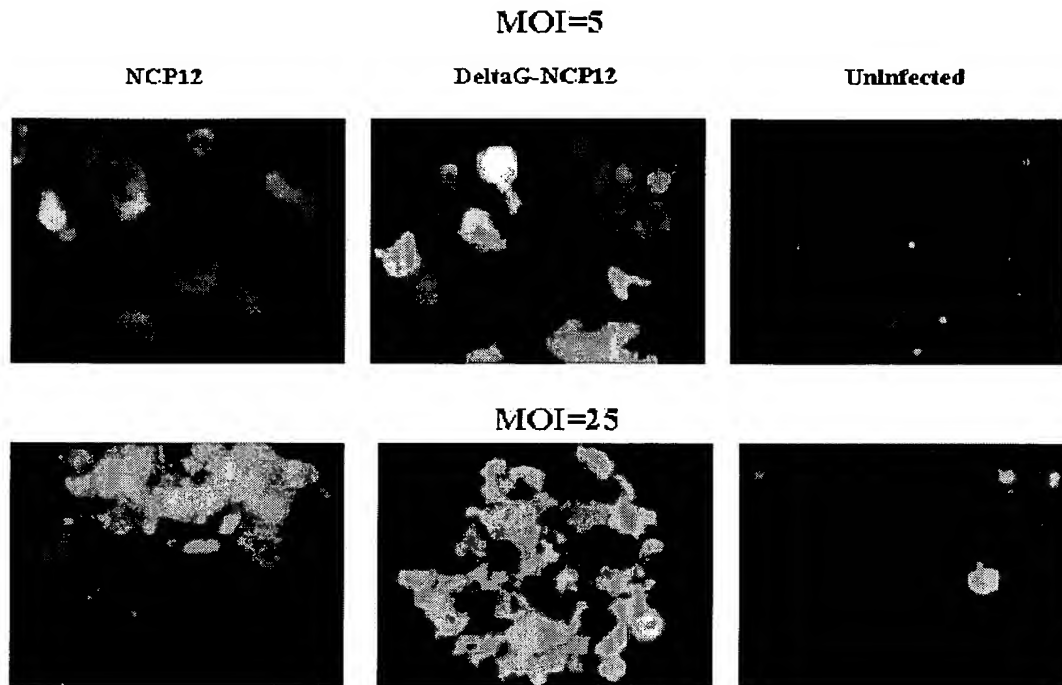


Figure 18

# Sample #176, 8 Days Post-infection

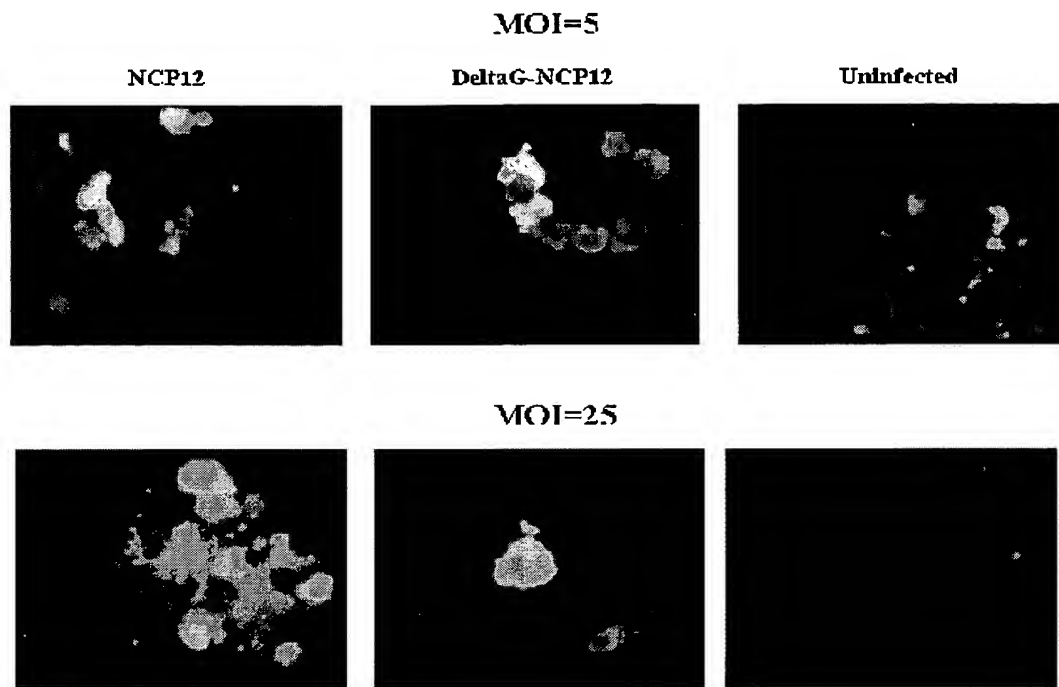


Figure 19

[illegible]

462]

H452A(B-A)  
G456D(G-D)  
W457A(W1A)  
R458A(F-A)  
W461A(W2A)  
W457A,W461A(WW-AA)  
G456D,W457A(DA)  
W457A,R458A,W461A(AA)  
G456D,W457A, W461A(DA)

[illegible]

Figure 21

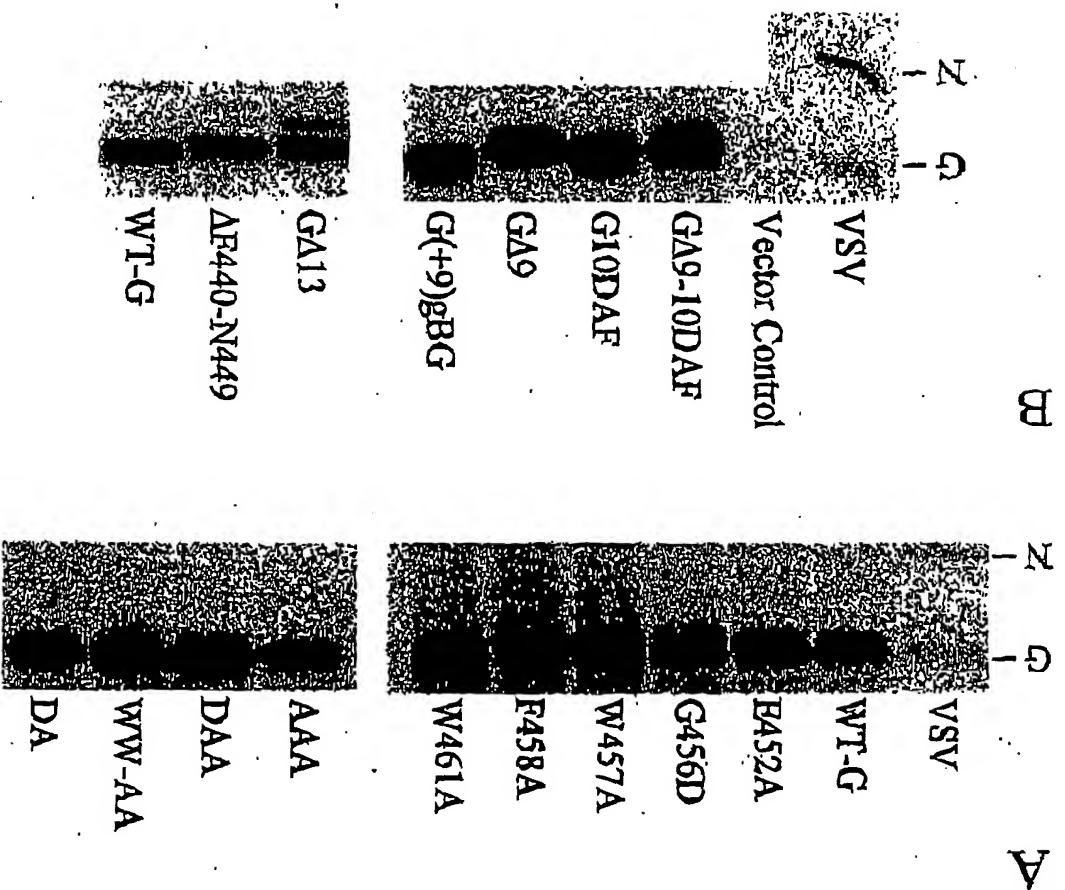
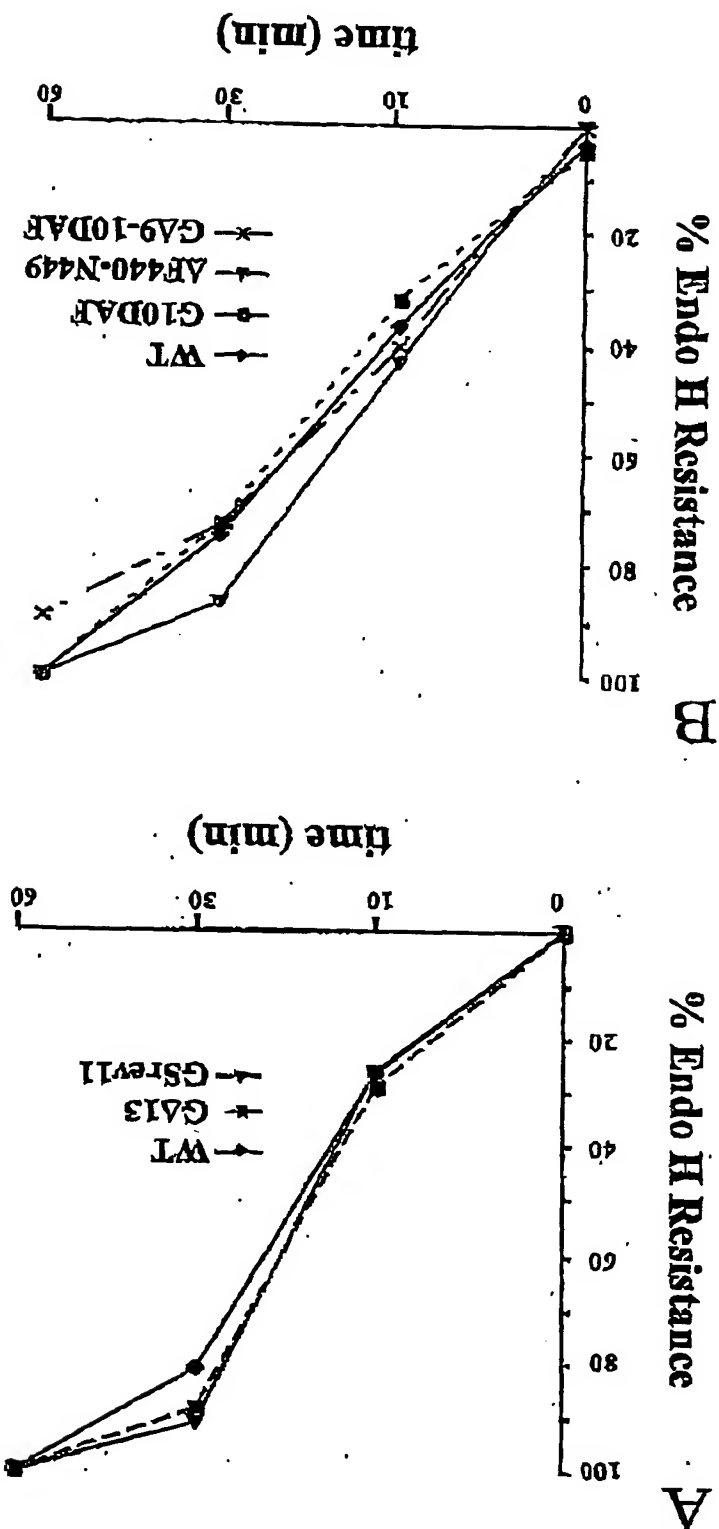
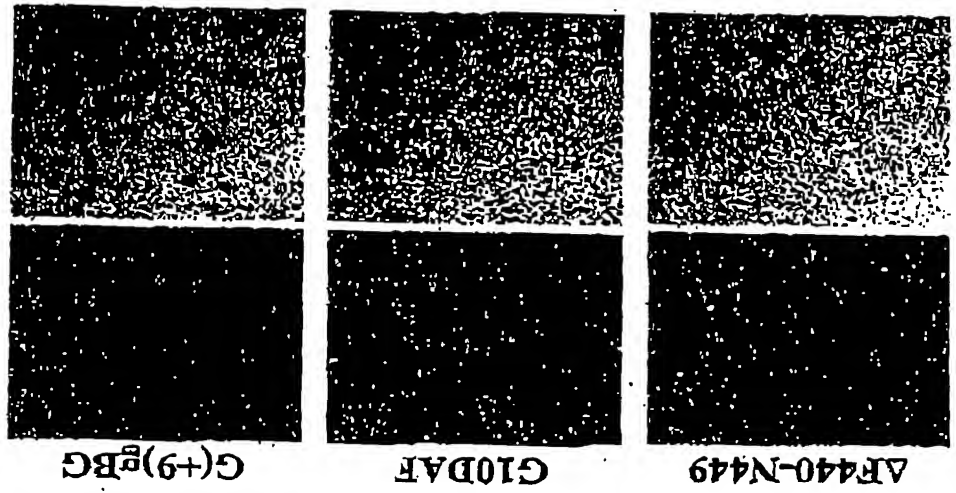


Figure 22

Figure 23



B



A

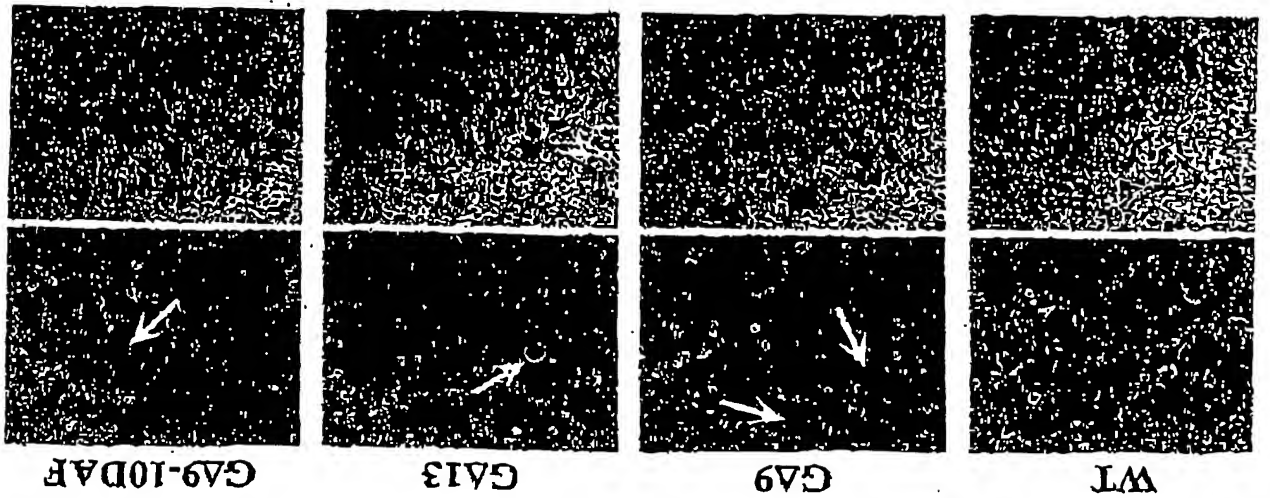


Figure 24

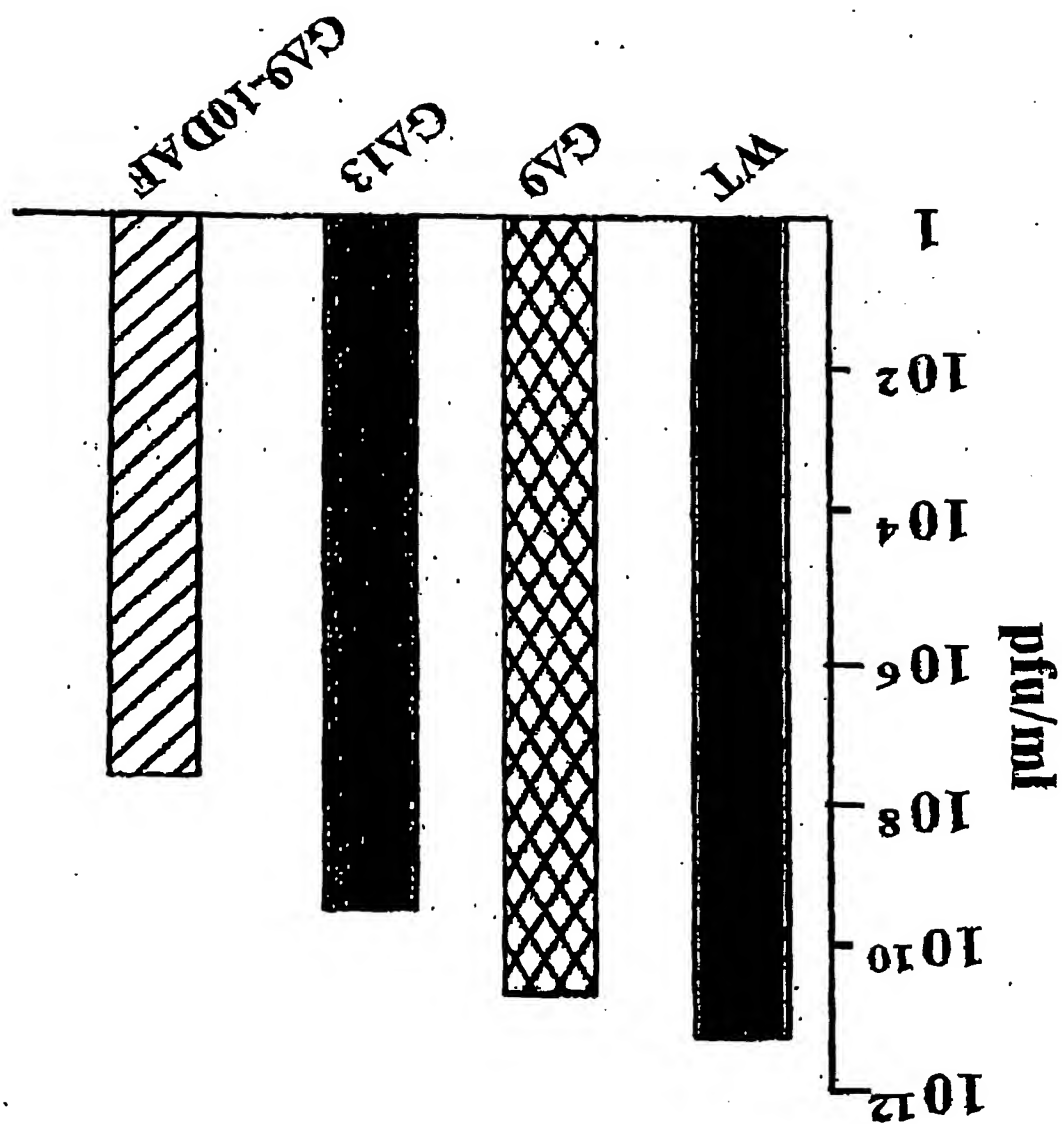


Figure 25

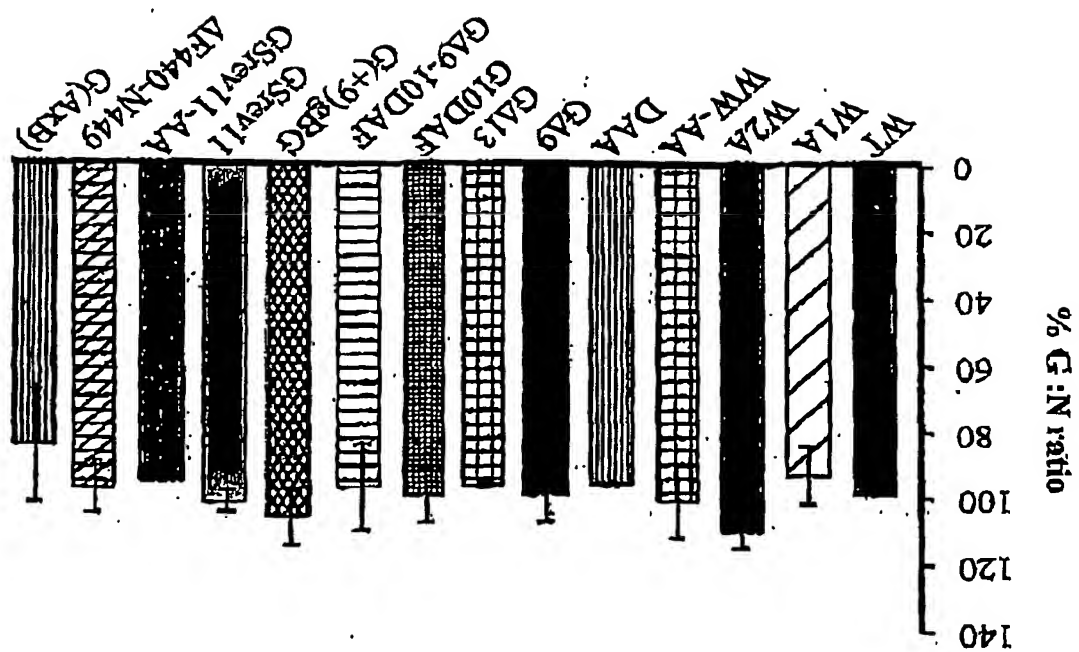
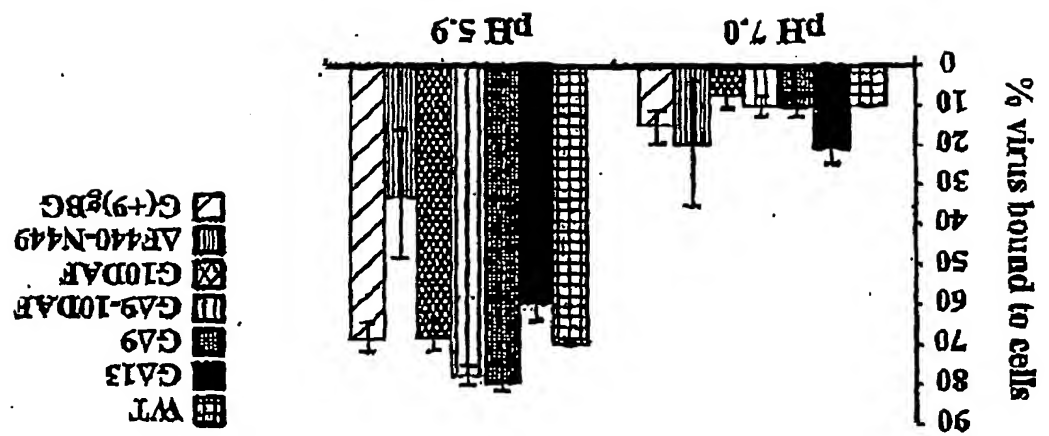


Figure 26

Figure 27



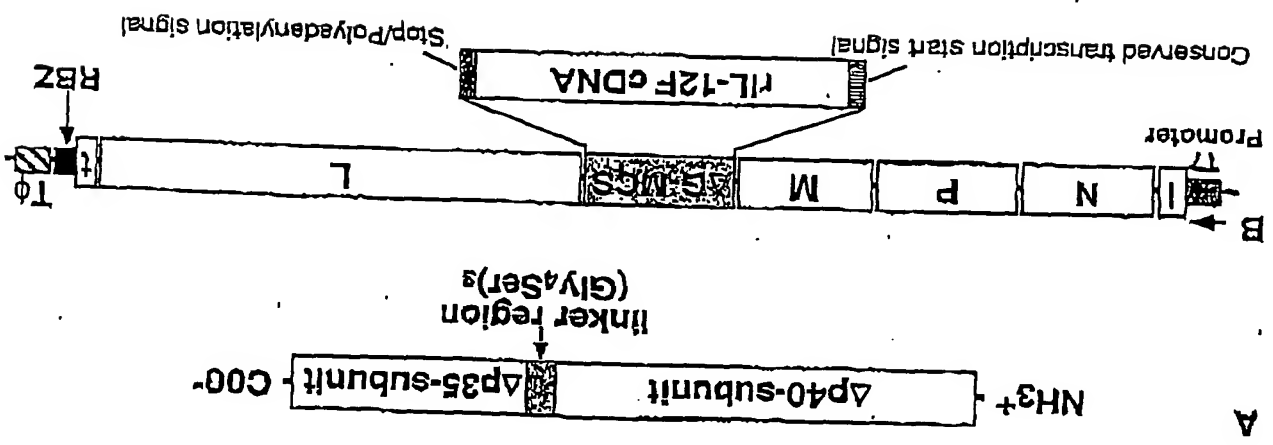


Figure 28

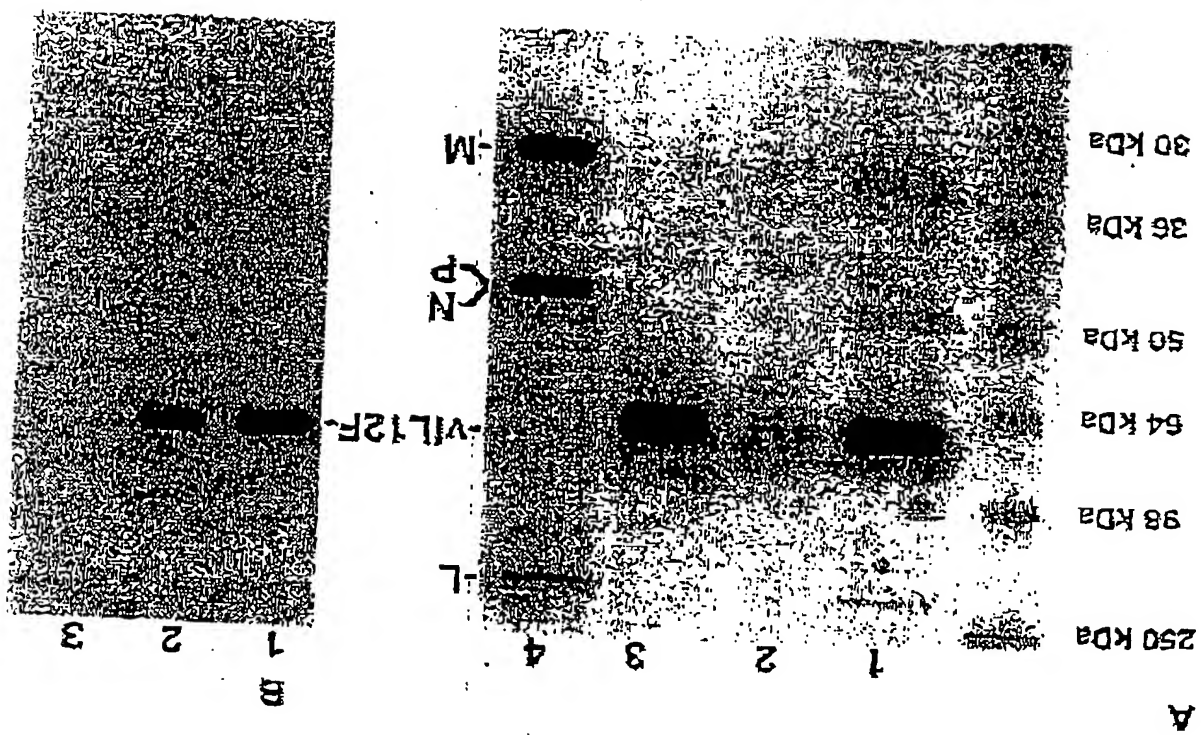


Figure 29

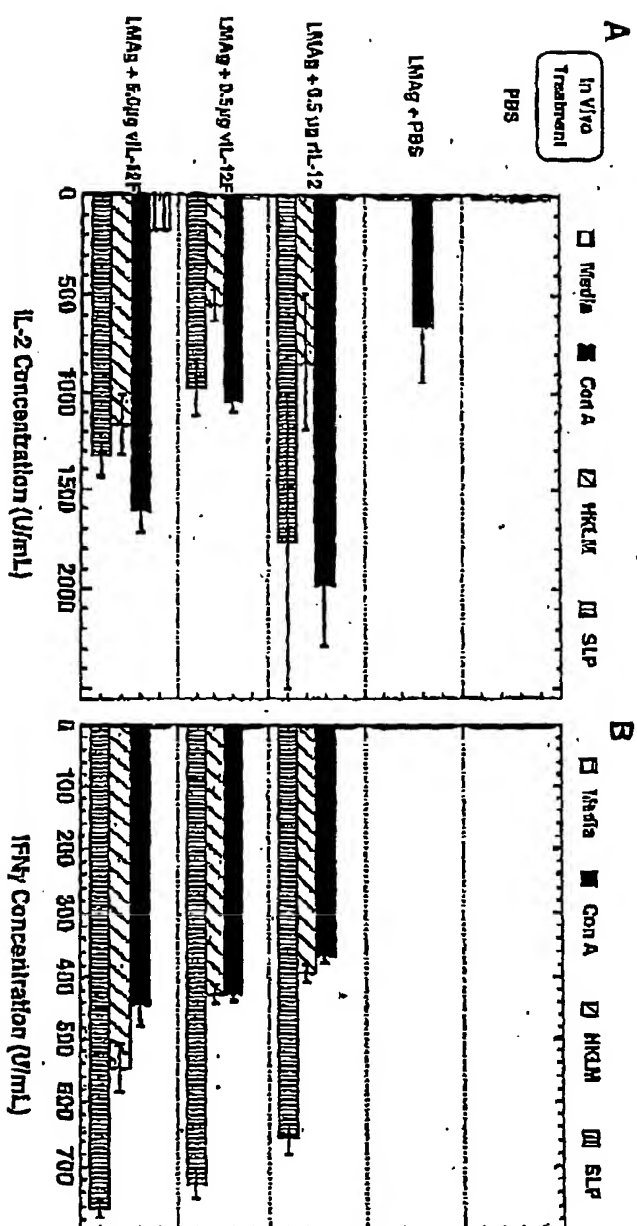
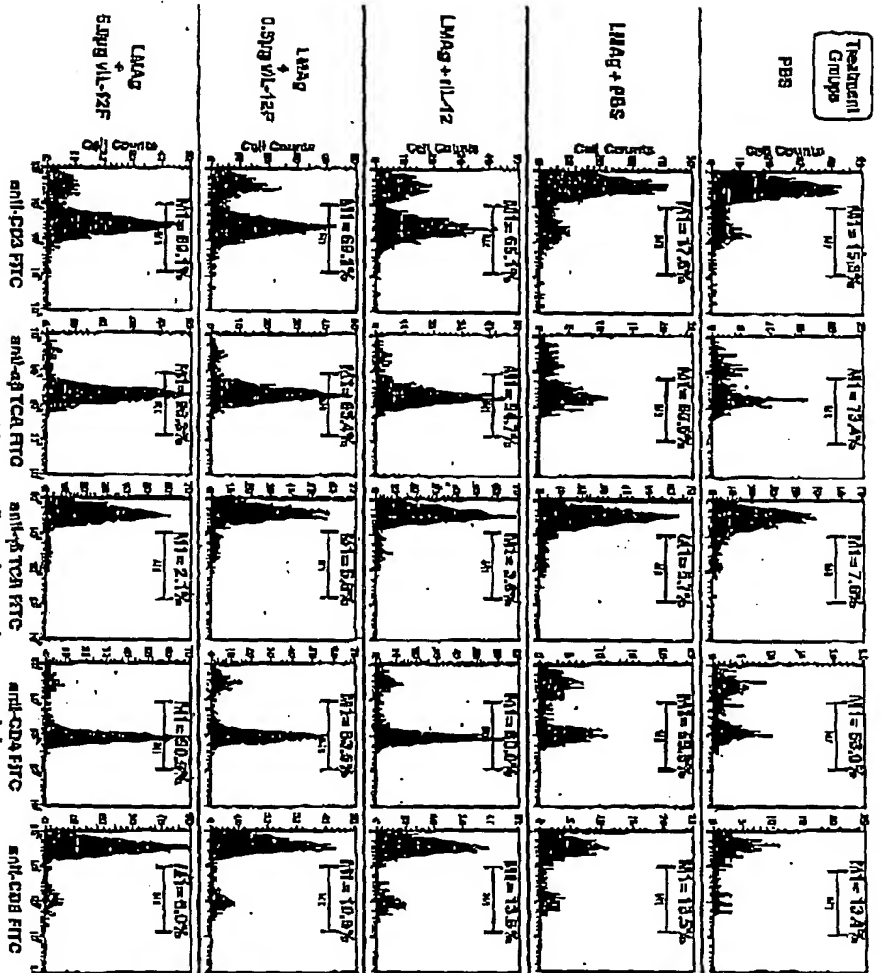


Figure 30

37



**Figure 31:**

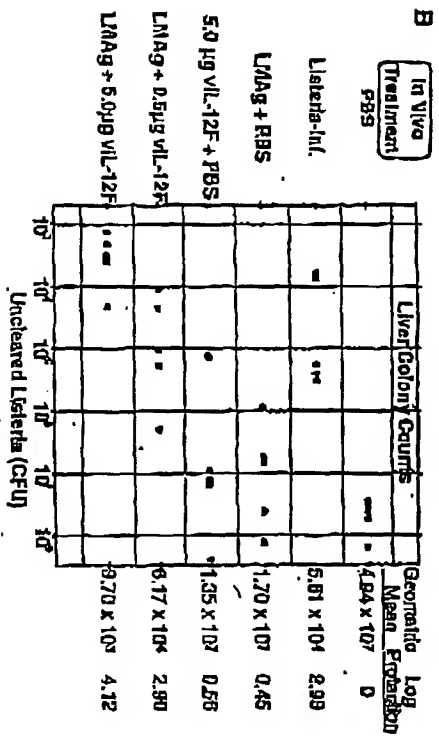
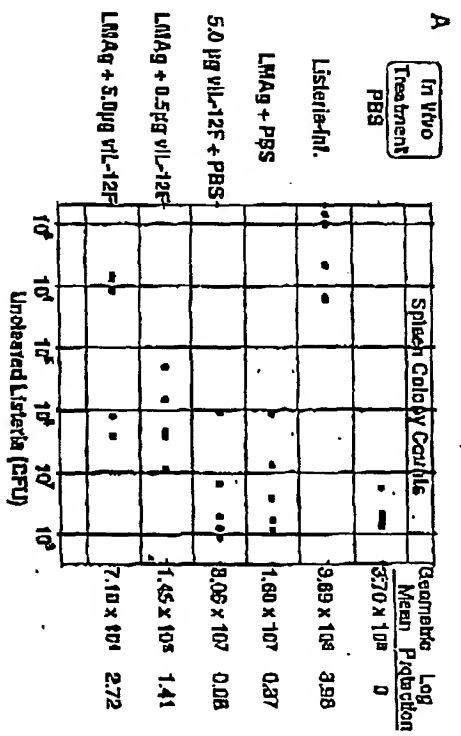


Figure 32

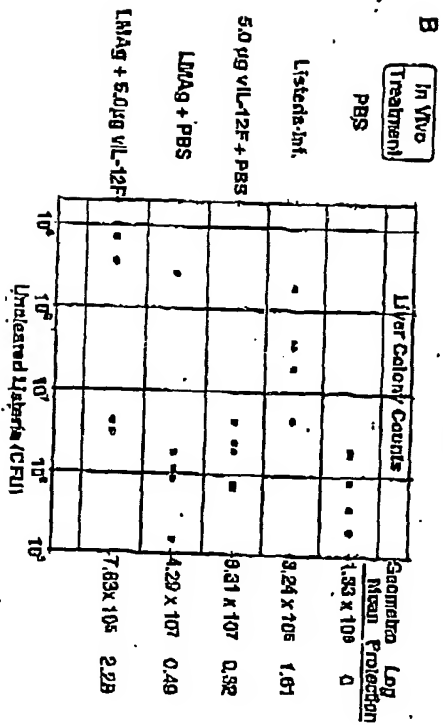
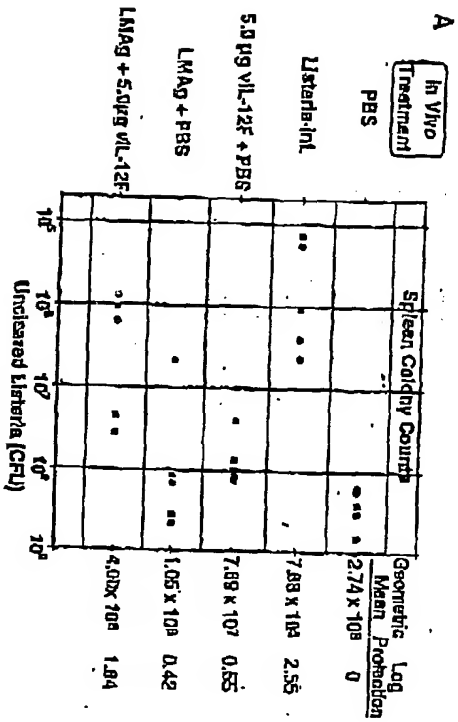
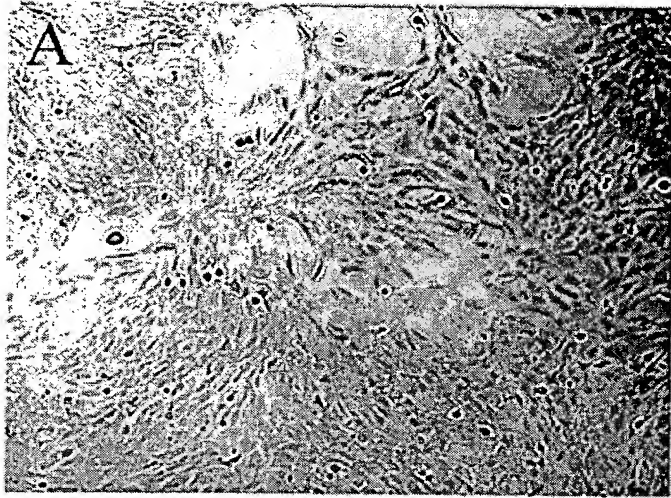
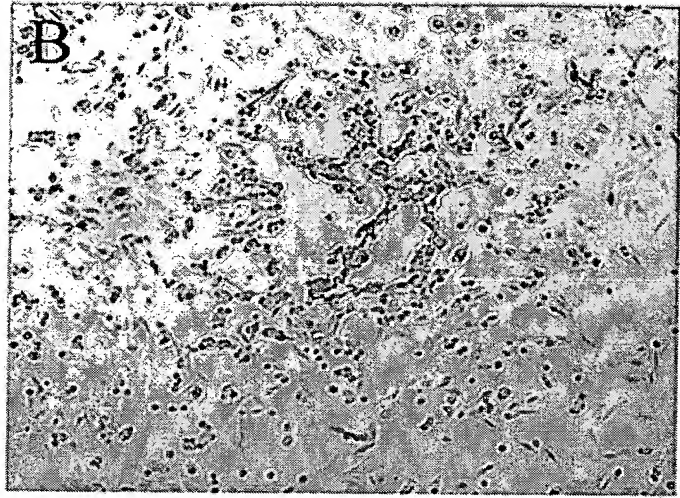


Figure 33

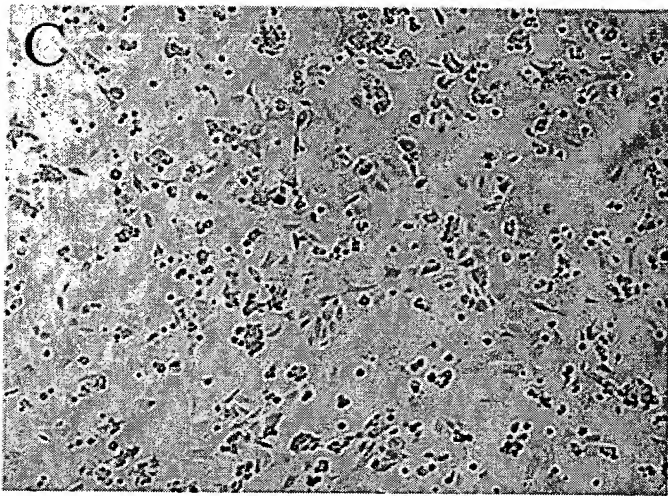
0 hr



10 hr



24 hr



48 hr

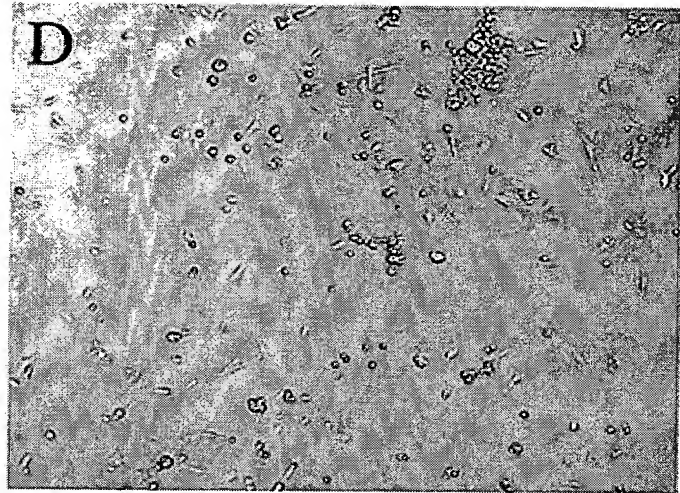


Figure 34

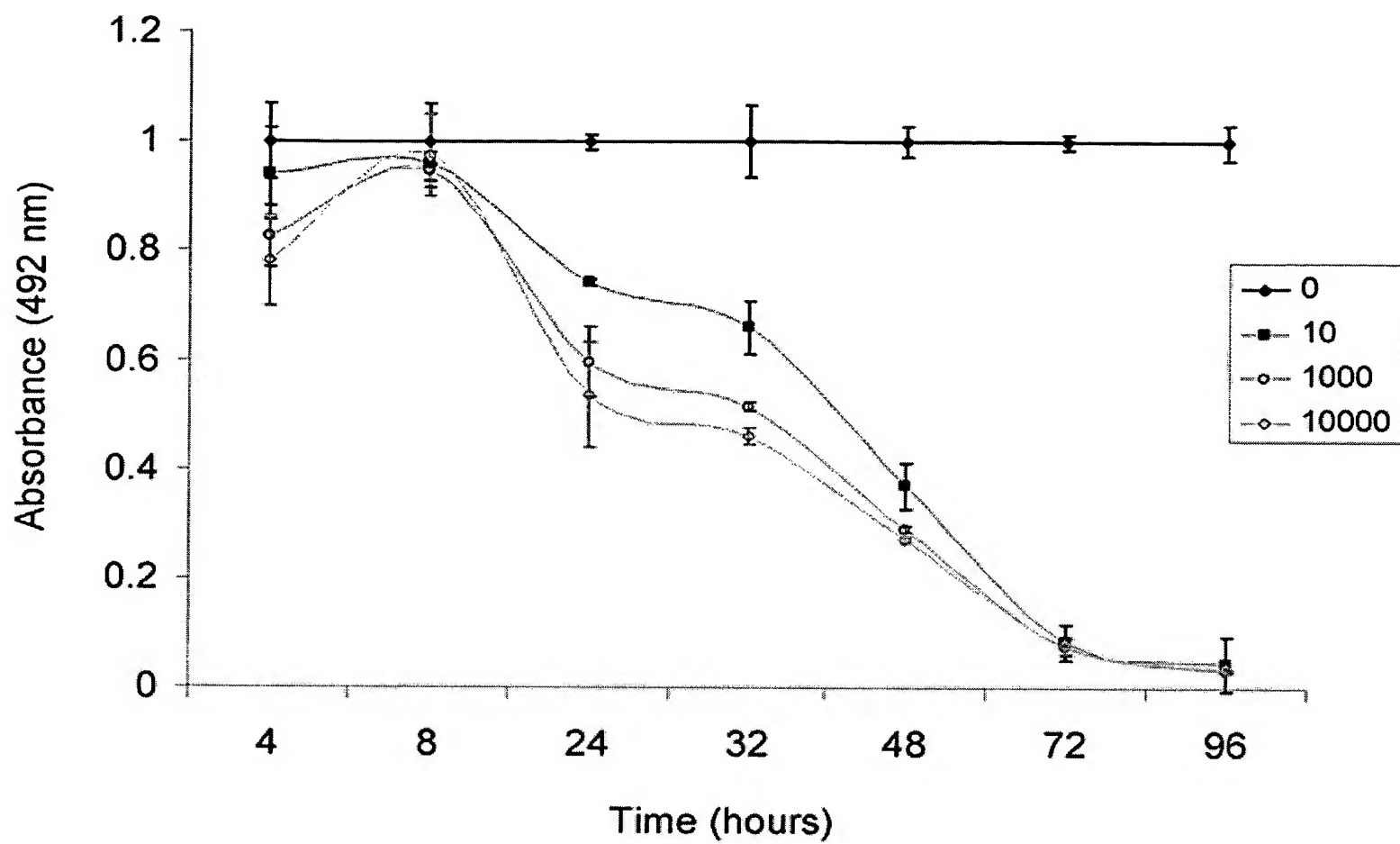


Figure 35

A

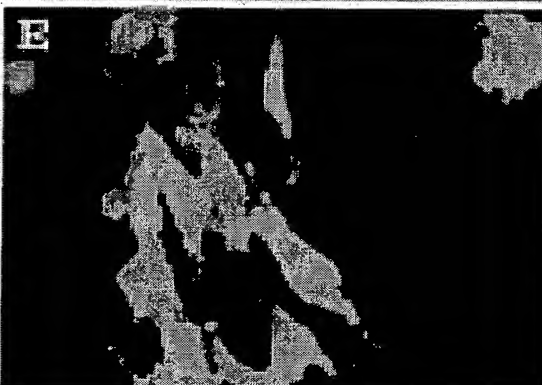
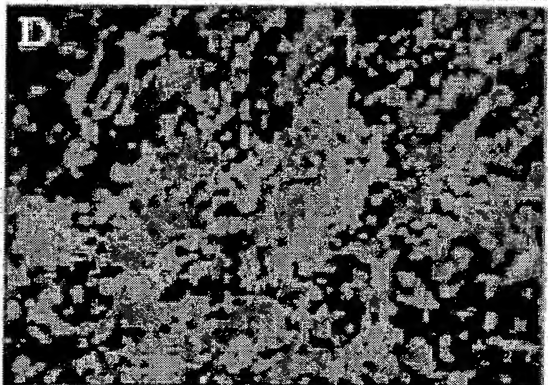
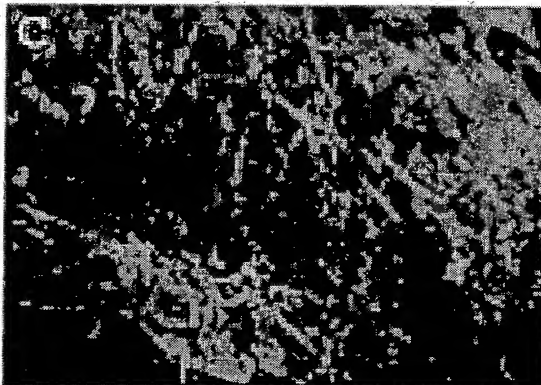
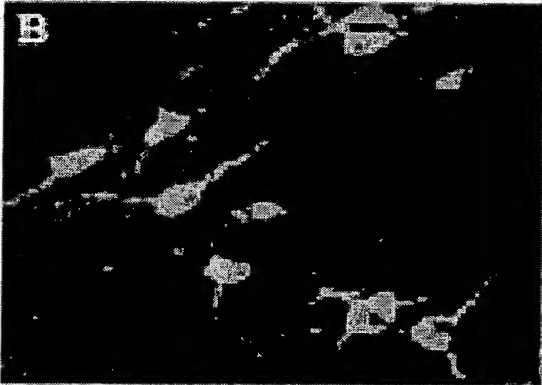
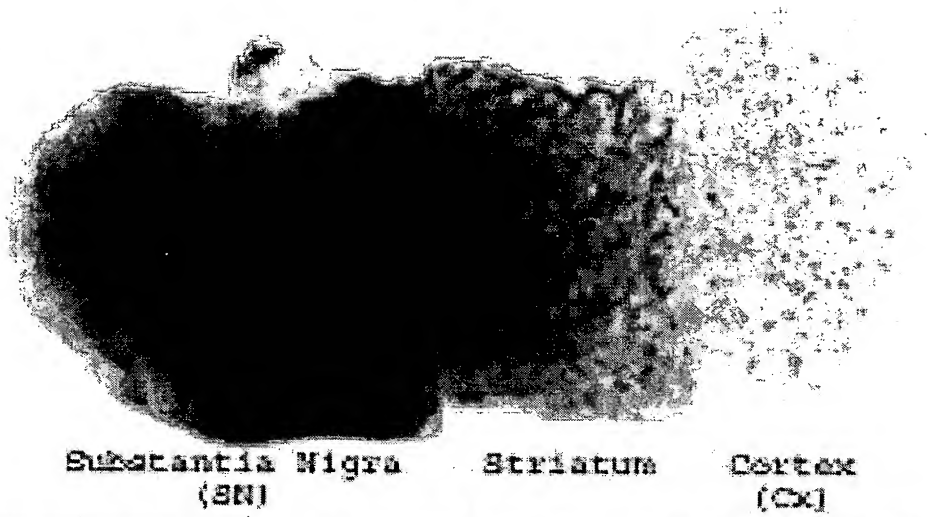


Figure 36

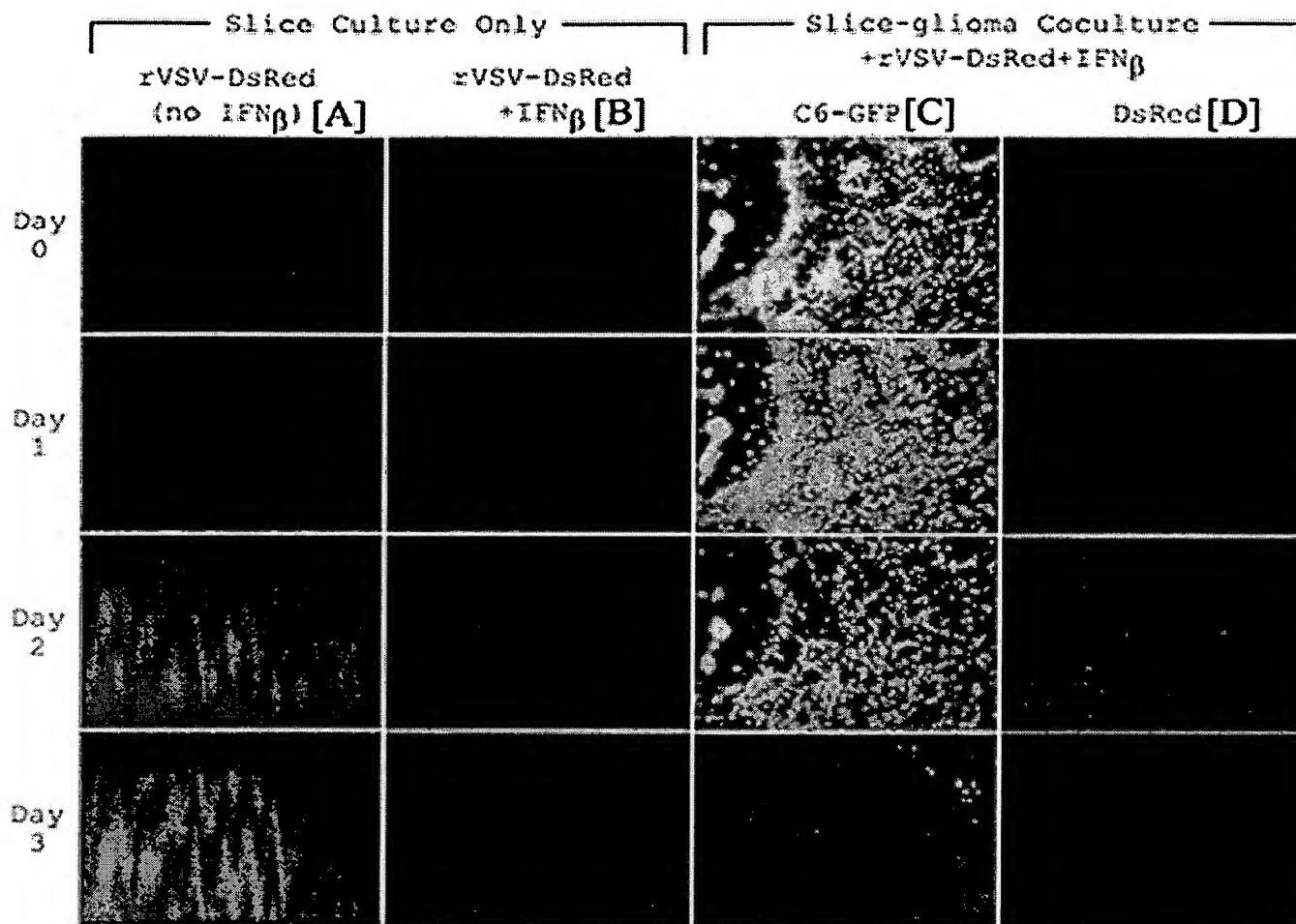
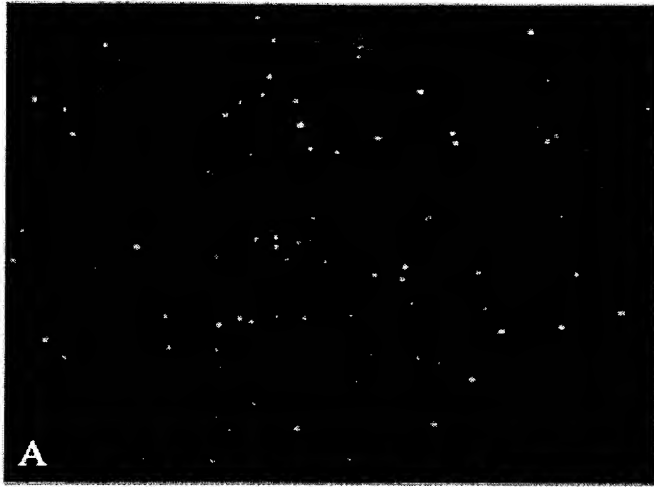


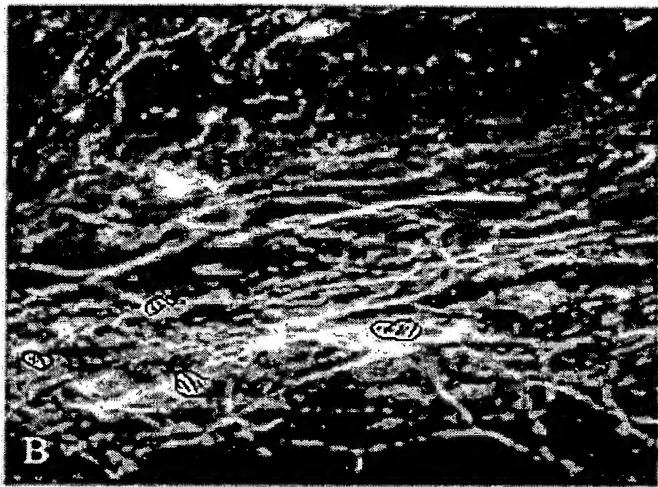
Figure 37

VSV-wt w/ no IFN

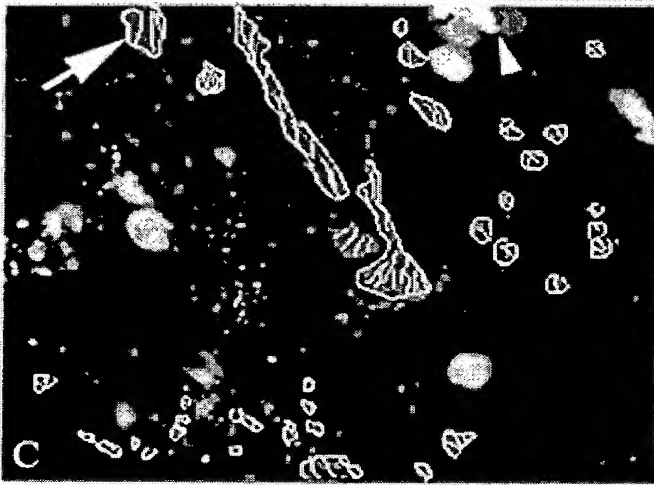


A

VSV-wt w/ 1,000u IFN

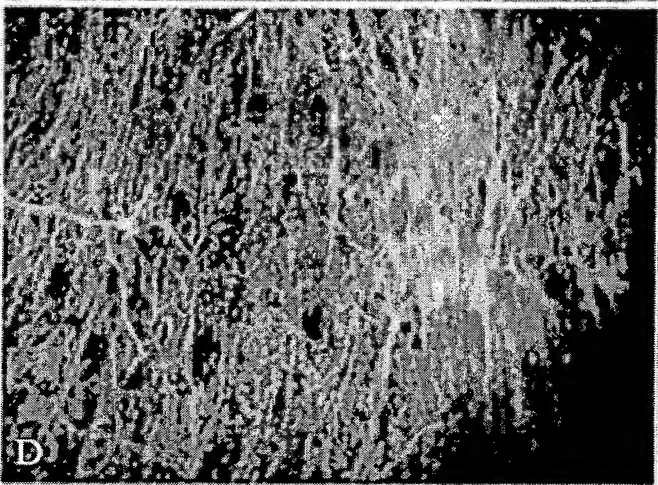


B



C

VSV-wt w/ 1,000u IFN on C6 co-cx



D

normal slice w/ IFN only

Figure 38

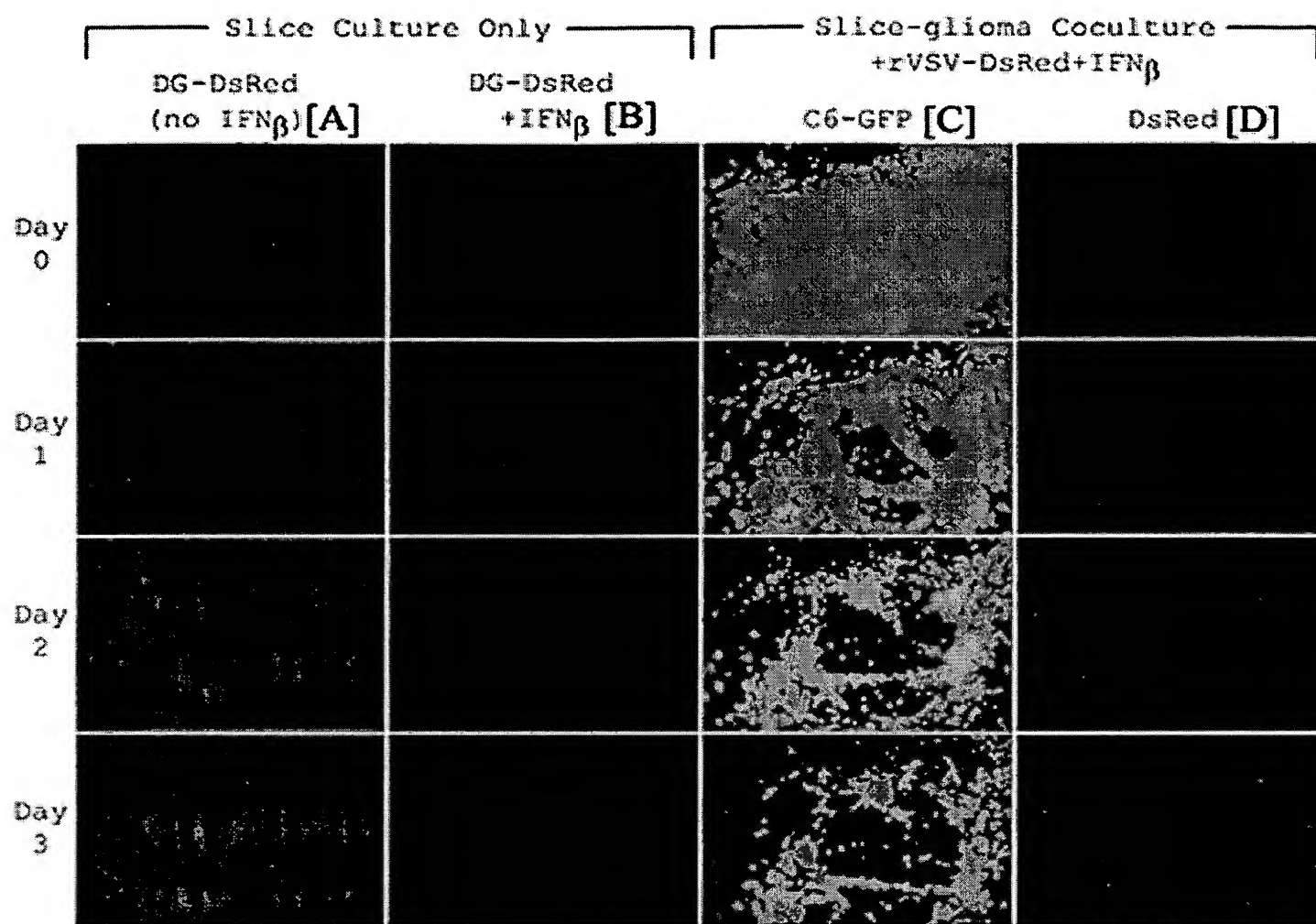
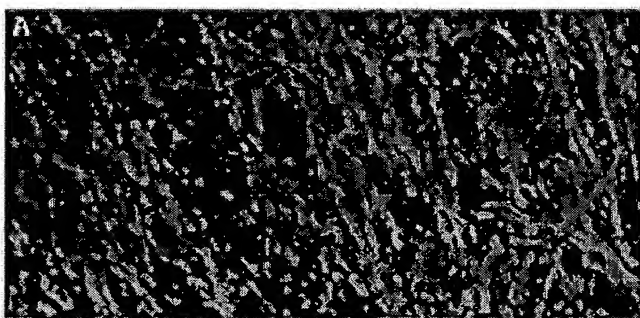
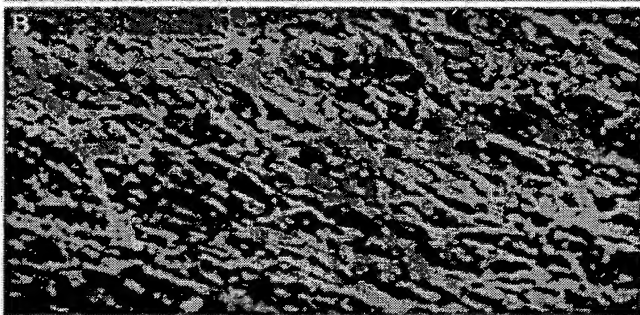


Figure 39

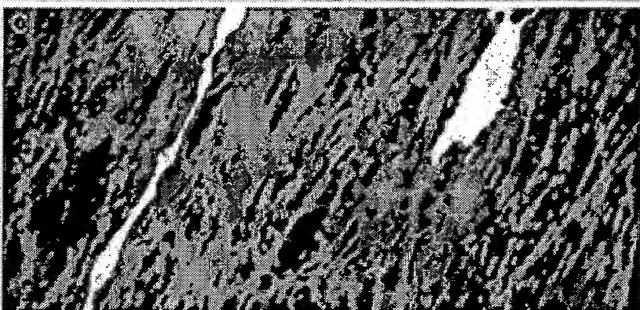
MAP2 Immunohistochemistry



DG-DsRed  
(no IFN $\beta$ )



DG-DsRed  
+IFN $\beta$



DG-DsRed  
+IFN $\beta$

Slice Culture  
Only

Slice-glioma  
coculture

Figure 40

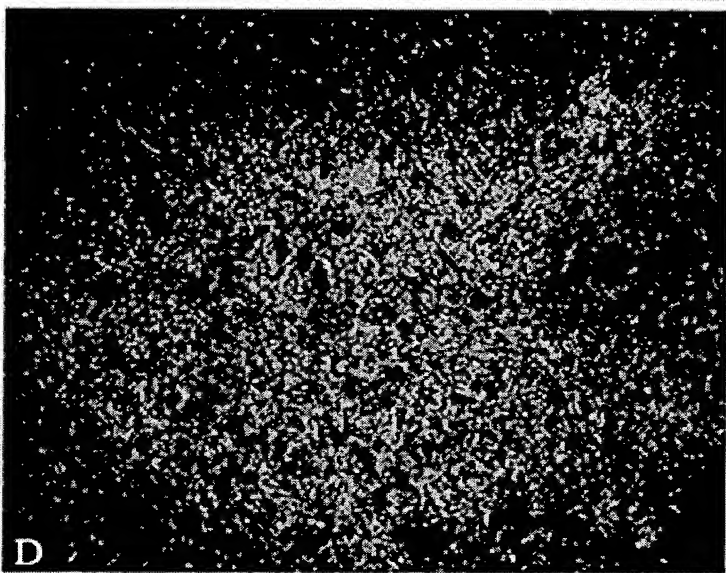
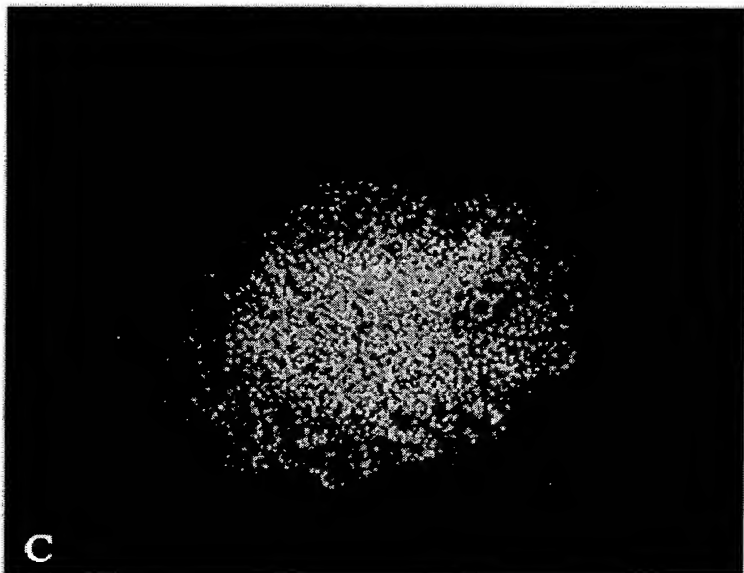
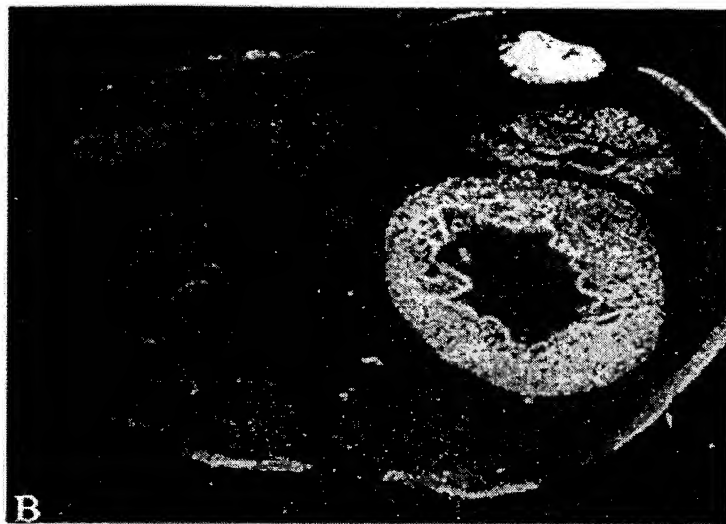
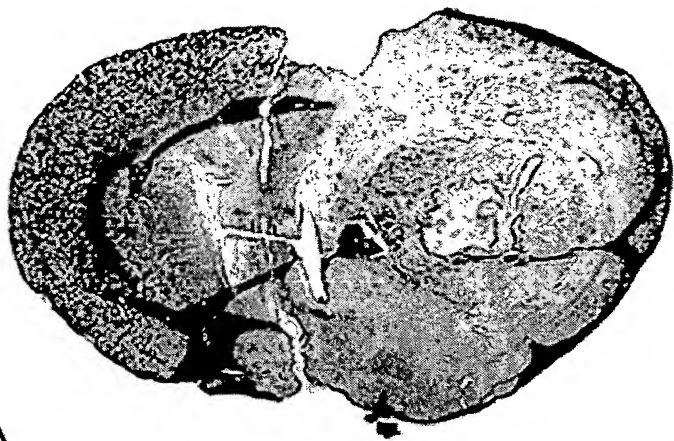


Figure 41

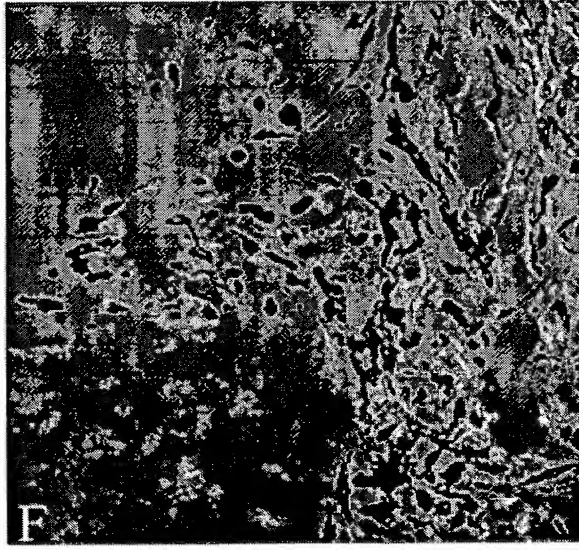
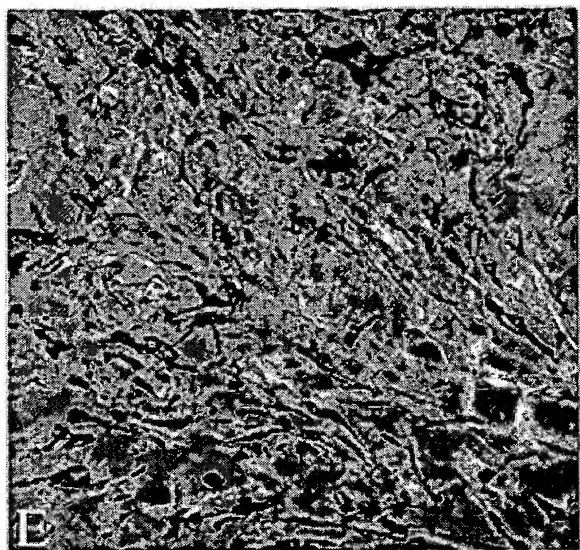
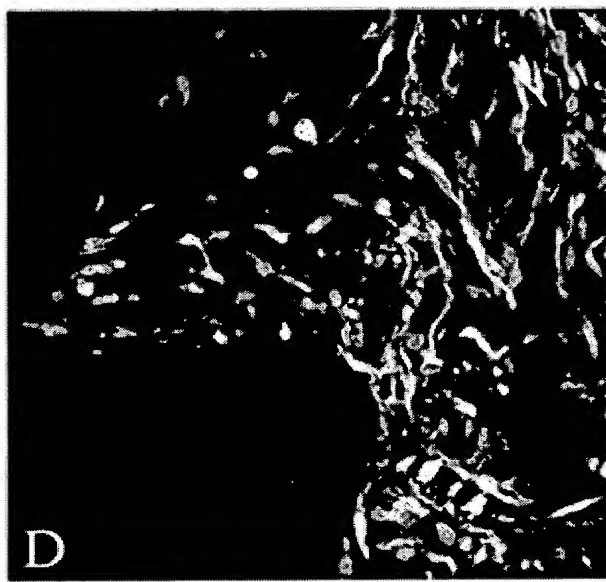
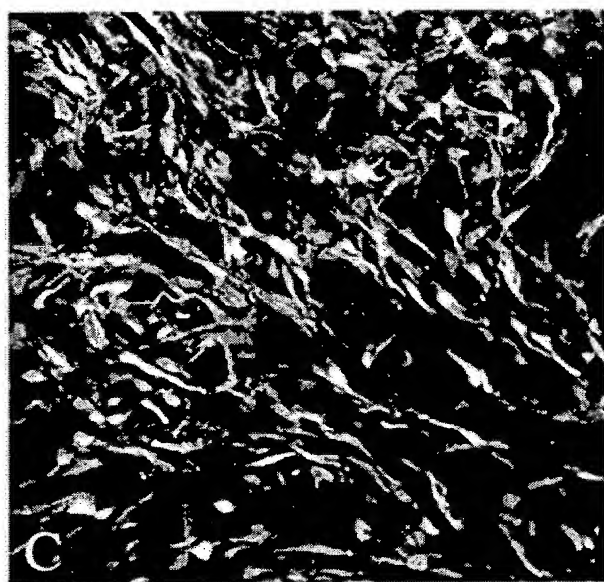
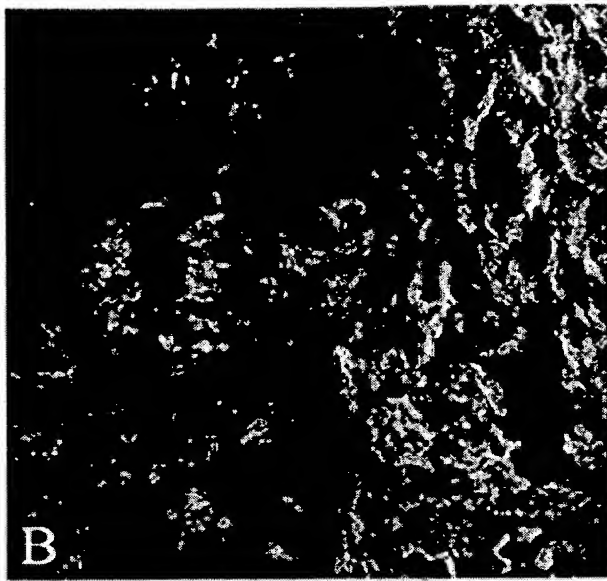
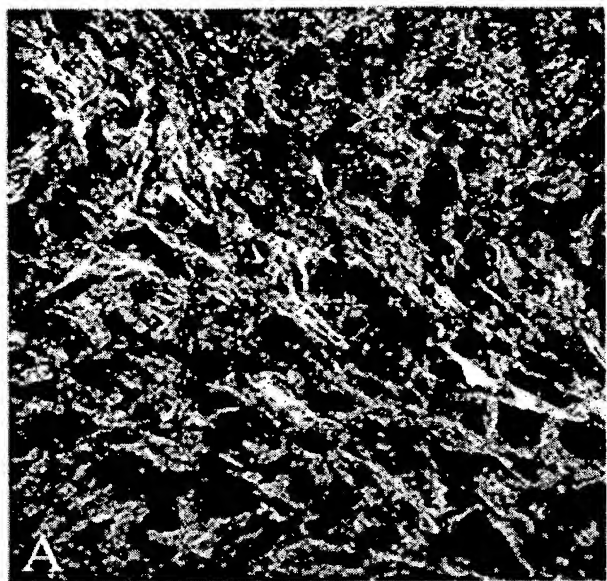


Figure 42